THE NATIONAL ACADEMY OF SCIENCES, INDIA

(Registered under Act XXI of 1860)

Founded 1930

Council for 1960

President

Dr. M. S. Randhawa, M.Sc., D.Sc., F.N.I., F.N.A.Sc., I.C.S., New Delhi

Vice-Presidents

Prof. K. Banerjee, D.Sc., F.N.I., F.N.A.Sc., Calcutta
Prof. P. S. Gill, M.S., Ph.D., F.A.P.S., F.N.I., F.N.A.Sc., Aligarh

Honorary Treasurer

Prof. S. Ghosh, D.Sc., F.R.I.C., F.N.I., F.N.A.Sc., Allahabad

Foreign Secretary

Prof. B. N. Prasad, Ph.D., D.Sc., F.N.I., F.N.A.Sc., Allahabad

General Secretaries

Dr. R. N. Tandon, M.Sc., Ph.D., D.I.C., F.A.Sc., F.N.A.Sc., Allahabad
Dr. K. N. Mathur, D.Sc., F.Inst.P., F.N.A.Sc., New Delhi

Members

Prof. N. R. Dhar, D.Sc., F.R.I.C., F.N.I., F.N.A.Sc., Allahabad
Prof. A. K. Bhattacharya, D.Sc., F.R.I.C., F.N.A.Sc., Sagar
Prof. S. Ranjan, M.Sc., D.Sc., F.N.I., F.N.A.Sc., Allahabad
Dr. K. S. Krishnan, D.Sc., F.R.S., F.N.I., F.N.A.Sc., New Delhi
Prof. P. L. Srivastava, M.A., D.Phil., F.N.I., F.N.A.Sc., Allahabad
Dr. L. A. Ramdas, M.A., Ph.D., F.N.I., F.N.A.Sc., New Delhi
Prof. M. D. L. Srivastava, D.Sc., F.N.A.Sc., Allahabad
Dr. S. H. Zaheer, M.A., Dr. Phil. Nat., F.N.A.Sc., Hyderabad-Dn.
Dr. A. C. Joshi, D.Sc., F.N.I., F.N.A.Sc., Solan

STUDIES ON STAPHYLOCOCCI WITH PARTICULAR REFERENCE TO STRAINS FROM BOVINE UDDER

I.—Biochemical activities*

By

B. S. MALIK, B.V.Sc. & A.H., M.V.Sc.; *and* C. M. SINGH, M.Sc., Ph.D.

Department of Pathology and Bacteriology, U. P. College of Veterinary Science and Animal Husbandry, Mathura

[Received on 10th November, 1959]

Various authors tried to differentiate pathogenic and non-pathogenic strains of Staphylococci on the basis of reactions shown by them in certain media. Gordon (1903-4), was first to differentiate strains from pathological sources on the basis of mannitol fermentation. Later on Hine (1922), Dudgeon and Simpson (1928), Hallman (1937), Julianelle (1937), Flaum (1928) and others confirmed the value of mannitol fermentation in differentiating pathogenic from non-pathogenic strains. Winslow *et al.* (1920) on the other hand reported lactose fermentation a more useful guide for the differentiation of pathogenic strains of this organism. Most of the workers considered all other sugars to be less reliable and noted, that very few sugars agree in giving identical reactions, although pathogenic strains on the whole had greater fermentative capacity than non-pathogenic strains.

The utility of various other substances was less marked in differentiating pathogenic and non-pathogenic strains of Staphylococci. Changes in litmus milk were studies by Dudgeon (1908), Winslow *et al.* (1920), Dupont (1931), Minett (1936), Cruickshank (1937), Smith (1947) and Schalm and Woods (1953) and found no definite correlation between these two types of strains.

Gupta and Chakravarti (1954), observed an absolute correlation between Methyl Red (M.R.) and Voges-Proskauar (V.P.) tests among 400 strains of Staphylococci but found no absolute relation with their pathogenicity.

Andrewes and Gordon (1905-6), reported H_2S production in smaller quantities by pathogenic Staphylococci than by non-pathogenic ones.

Hucker (1924), noted the power of nitrate reduction more in aureus strains than albus strains.

Fusillo and Jaffurs (1955), found negative correlation between coagulase and urease production of Staphylococci except in few instances.

Barber et al. (1951) anh Barber and Kuper (1951) found a close correlation between coagulase production and phosphatase production among different strains of Staphylococci which was confirmed by Chiarolanza (1954) and Lovell (1958). Gupta and Chakravati (1954), on the other hand did not find absolute correlation between coagulase and phosphatase tests.

Gelatin liquefaction test also gave variable results in the hands of different workers, although pathogenic strains possessed most of the capacity.

MATERIALS AND METHODS

Strains.—One hundred and seventy eight strains of Staphylococci, comprising 167 from bovine normal and abnormal milk samples, 10 from caprine mastitis cases and one from a case of ovine abscess, were studied. Of these 125 strains were isolated at Mathura, 15 were obtained from Lucknow, 29 were received from Madras and rest 9 were supplied from Calcutta. On the basis of pigment production* 53 strains were golden yellow, 10 off-coloured, 87 white and 28 lemon yellow.

Sugar fermentation.—In all, 20 substrates as indicated in table I, were tested. Strains were grown in peptone water containing 1% of the substance and Andrade's indicator. The results were noted daily for three days at 37°C.

Changes in Litmus milk.—Three drops af each of the six hour peptone water culture, were added to each litmus milk tube. Incubation was done at 37°C. for three days and left at room temperature for further 11 days. Changes were recorded on daily observations.

Methyl-Red (M.R.) test.—To 5 ml. of a three day old culture of glucose phosphate peptone water, 5 drops of methyl red solution (0·04 %) was added. Red colour was taken as positive and yellow as negative.

Voges-Proskauer (V.P.) test.—Barritt's modification as cited by Wilson and Miles (1955) was used.

Indole production.—Using Bohme's reagent and Happold and Hoyle's modification was adopted. (Wilson and Miles, 1955).

Ammonia production.—Tested on peptone water culture, after growing for three days at 37° C and by adding Nessler's reagent. Brown colour was taken as positive and yellow as negative.

---

*For the purpose of description in the text, golden yellow, white and lemon yellow strains were considered aureus, albus and citreus respectively.

Hydrogen sulphide test.—Cultures were grown on liver agar slants, with a strip of filter paper soaked in 10 % lead acetate solution attached in between the cotton plug and the tube. Browning or blackning of the paper was taken as positive.

Nitrate reduction.—Strains were tested in potassium nitrate medium after growing for three days at 37° C, with reagents recommended by Griess Ilosvay. (Wilson and Miles, 1955.)

Urea hydrolysis.—Urease test was read in the medium as recommended by Merchant and Packer (1956). Readings were recorded at 24 hourly intervals till the third day.

Methylene blue reduction.—Tested on a 24 hour broth culture at 37° C after adding one drop of 1 % acquous methylene blue solution. The cultures were further incubated at 37° C and results noted. Decolorization of the culture was indication of positive result.

Phosphatase test.—One per cent phenolphthalein diphosphoric acid was sterilized by Seitz filtration. One ml. quantity of this solution was added to each 99 ml. of melted nutrient agar to give a final concentration of 0 01 %. Plates were poured and streaked after drying the surfaces. After 24 hour incubation at 37° C the colonies of positive strains turned pink when exposed to ammonia vapours.

Gelatin liquefaction.—Two drops of each of the 18 hour broth cultures, were added in gelatin medium (20 %). The tubes were incubated at 37° C for seven days. The liquefaction was recorded after leaving the tubes at 4° C along with uninoculated control for half an hour. Tubes showing no solidification of the medium were indicative of positive results.

RESULTS

Most of the bovine strains tested, produced variable fermentation reactions in different sugar media. Rhamnose, dulcitol and adonitol were not fermented by any strain. Arabinose, xylose, raffinose and inositol were fermented by occasional strain. The capacity of the strains to ferment salicin was also very limited. Glucose, fructose, galactose, mannose, lactose, sucrose, inulin, mannitol and maltose were fermented by most of the strains (Table I).

Most of the aureus strains fermented mannitol within 24 hours. Albus and citreus strains fermented this substance to a lesser extent, but with an increased period of incubation the number of positive reactions increased to a considerable extent.

None of the caprine strains fermented arabinose, rhamnose, xylose, inositol, adonitol, dulcitol, salicin and raffinose. Only one strain fermented dextrin and all strains fermented mannitol, glucose, lactose, inulin, galactose, mannose, fructose and trehlose. Only two of the caprine strains fermented maltose.

Out of 137 bovine strains tested in litmus milk, 18 (13 %), produced acid ; 2 (I·4 %) acid and clot ; 103 (75·2 %), acid, clot and reduction ; 2 (1·4 %), alkalinity and 5 (3·7 %), alkalinity and Peptonization. Practically all clots contracted and 36 out of these expressed clear whey at the tops of the clots or on the sides. In most of the tubes reactions changed to acid side within first 24 hours and clots were formed within next twenty-four hours of incubation. Practically all types of changes were noticed but the most abundant type being acid, clot and reduction.

The correlation of pigment production and changes produced in litmus milk are shown in table II below.

TABLE I

Bovine strains showing correlation of pigment production and sugar fermentations

Substances	Aureus		Albus		Citreus	
	+	—	+	—	+	—
Arabinose	3	50	7	79	0	28
Xylose	2	51	3	83	0	28
Rhamnose	0	53	0	86	0	28
Glucose	51	2	60	3	25	3
Fructose	52	1	61	2	25	3
Galactose	52	1	59	3	21	7
Mannose	52	1	61	1	23	5
Maltose	51	2	60	26	7	21
Lactose	51	2	48	15	18	10
Sucrose	52	1	61	2	25	3
Trehlose	19	0	41	26	16	8
Raffinose	0	53	2	55	1	27
Inulin	51	2	47	6	23	5
Dextrin	38	15	39	24	19	9
Mannitol	47	6	61	25	25	3
Dulcitol	0	53	0	86	0	28
Inositol	0	53	1	62	1	27
Adonitol	0	53	0	63	0	28
Sarbitol	37	16	18	45	3	25
Salicin	5	48	5	58	0	28

 = Acid but no gas.
— = No reaction.

TABLE II

Bovine strains showing correlation of pigment production and changes in the litmus milk.

Pigment	Changes						
	N	A	A. C.	A. C. R.	Al.	Al. P.	Total
Aureus	1	2	1	45	..	..	49
Albus	2	6	..	46	1	5	60
Citreus	4	10	1	12	1	..	28

N	= No change.	A. C. R.	= Acid, clot and Reduction.
A	= Acid.	Al.	= Alkalinity.
A. C.	= Acid and Clot.	Al. P.	= Alkalinity and peptonization.

It is observed from table II that the percentage of intensity of reactions decreased gradually from aureus strains to citreus. No alkaline reaction was shown by any of the aureus strains ; five of the albus strains showed alkalinity and peptonization.

Among caprine strains, 9 showed acid. clot and reduction and expressed clear whey on the tops of the clots. The remaining one strain showed acid reaction only. Solitary strain of ovine origin showed acid, clot and reduction.

Out of 143 strains tested for M. R. test, 126 (83·1 %), gave positive and 17 (11·9 %) negative results. Aureus strains possessed highest and citreus lowest capacity to show M. R. positive reaction (Table III).

TABLE III

Strains showing correlation of biochemical reactions and pigment production

Pigment	M.R.		V.P.		Indole		Ammonia production		H₂S production		Nitrate reduction		Urea hydrolysis		Methylene blue reduction		Phosphatase production		Gelatin liquefaction	
	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+	—
Aureus	48	1	45	4	0	49	25	2	17	32	48	1	47	2	49	0	45	8	50	
Off-coloured	8	2	8	2	0	10	5	5	8	2	10	0	10	0	9	1	8	2	10	03
Albus	52	4	52	4	0	56	1	55	53	3	40	16	37	19	51	5	35	51	53	33
Citreus	18	10	15	13	0	28	2	26	26	2	25	3	21	7	25	3	10	18	17	11

Out of 143 strains, 120 (83·9 %) gave V. P. positive reaction. In this test also the capacity of acetyl methyl carbinol production was possessed highest by aureus strains. The corre lation of M. R. and V. P. reactions was 95 %. All the 143 strains under study were negative to indole test. Only 33 (23·1 %), out of 143 strains liberated ammonia. About half of the aureus and off-coloured strains gave spositive test. H₂S production was shown more regularly by albus and citreus strains. Nitrate was reduced to nitrite by 86% of the strains. Nineteen out of 20 negative strains were from the albus and citreus strains.

Out of 143 strains, 115 (80·4 %), produced urease. All caprine strains were positive to this test. The reaction and percentage of positive strains increased with the length of incubation period. Ninety three per cent of the strains tested gave methylene blue reduction test.

Out of 177 strains tested for phosphatase production, 98 strains (55·4 %), gave positive reaction. Although most of the aureus strains possessed this property yet to a lesser extent albus and citreus strains also gave positive reaction.

Gelatin liquefaction was shown by 130 strains (73·4 %), out of a total of 167 strains. Out of 50 aureus strains 47 liquafied this substance. All the caprine strains gave positive results more quickly.

[105]

The correlation of biochemical tests and pigment production is shown in table III above.

DISCUSSION

Because of notorious diversity, fermentation reactions served no useful purpose in classifying this organism. Various workers stressed the importance of mannitol and lactose fermentation in distinguishing pathogenic from non-pathogenic strains.

In this study, although majority of the aureus strains fermented mannitol (88·7 %) and lactose (96·2 %), yet these substances were also acted upon by a good number of albus and citreus strains as well. (Table I). Cowan (1938), reported 94 % and 68 % correlation between golden yellow pigment and mannitol fermentation with human and animal strains respectively. Gwatkin (1937) found only 190 udder Micrococci, out of a total of 275 pathogenic ones fermenting mannitol. The results of Smith (1947) and Nakagawa (1958), with animal strains also indicated mannitol fermentation to be less reliable in distinguishing pathogenic from non-pathogenic strains. Hence the significance of mannitol fermentation with regard to animal strains seems to be ambiguous.

Similarly the reliability of phosphatase test (Gupta and Chakravarti, 1954) and Gelatin liquefaction (Edwards and Rippon, 1957) in differentiating pathogenic from non-pathogenic strains was doubtful. In the present investigation, in addition to aureus strains, quite appreciable number of albus and citreus strains also gave positive phosphatase test and liquefied gelatin which proved unreliability of these tests.

Gupta and Chakravarti (1954) found an absolute correlation between M. R. and V. P. tests. These authors indicated that production of acetyl methyl carbinol did not appear to be enough to neutralize the acidity produced and pH is maintained at a level where M. R. test is given. The correlation of M. R. and V. P. tests (Table III) in this work is found to be about 95 % which is very near to the results of Gupta and Chakravarti.

The findings of Hucker (1924), with regard to H$_2$S production are also confirmed.

The nitrate reduction results of aureus strains are also in conformity with Hucker (1924), but the results obtained with albus strains (40 %) are higher.

Fusillo and Jaffurs (1955), found negative correlation between coagulase and urease production. In the present study majority of the strains were positive to urease test.

The changes in litmus milk were in agreement with many other workers, exeepting few variations. The reaction of peptonization of acid clots, described by Winslow et al. (1920). was not observed.

SUMMARY AND CONCLUSIONS

Various biochemical studies were corried out on 178 strains isolated from normal and abnormal milk samples of bovine and caprine origin (one from a case of ovine abscess), representing different parts af India. Of these, 53 strains were golden yellow, 10 off-coloured, 87 white and rest 28 lemon yellow. Out of 20 sugars tested, rhamnose, dulcitol and abdonitol were not fermented by any strain ;

arabinose. xylose, raffinose, inositol and salicin were fermented by occasional strain; glucose, fructose, galactose, mannose. lactose, sucrose, inulin, mannitol, and maltose were fermented by most of the strains. The correlation of aureus coloured strains with mannitol and lactose fermentation was 88·7 % and 96·2 % respectively but the value of these substances in differentiating pathogenic from non-pathogenic strains, is doubtful, because an appreciable number of albus and citreus strains (coagulase negative) also fermented these sugars. Changes in litmus milk and other tests like M. R., V. P., ammonia production, nitrate reduction and Urea hydrolysis were given to a greater extent by aureus strains and the percentage decreased gradually towards albus and citreus strains. The realiability of Phosphatase test and gelatin liquefaction was also not substantiated. A positive correlation between M. R. and V. P. tests has been confirmed. The capacity of H_2S production was observed more in albus strains than in aureus ones.

REFERENCES

1. Andrewes. F. W. and Gordon, M. H., (1905-6). *Rep. Loc. Govt. Bd. Publ. Hlth. Suppl.* P. 543. (Quoted by Wilson and Miles, 1955).

2. Barber, M. and Kuper, S. W. A., (1951). *J. Path. Bact.* **63**, 65-68.

3. Barber, M., Brooksbank B. W. L. and Kuper, S. W. A., (1951.) *J. Path. Bact.* **63**, 57-64.

4. Chiarolanza, V., (1954). *Rev. 1st Sierster ital.* **29**, 27-33. (Brit. Abst. Med. Sci. 1955, 2, 95.)

5. Cowan, S. T., (1938). *J. Path. Bact.* **48**, 169-173.

6. Cruickshank, R., (1937). *J. Bact.* **45**, 295-303.

7. Dudgeon, L. S., (1908). *J. Path. Bact.* **12**, 242-257.

8. Dudgeon, L. S. and Simpson, J. W. H., (1928). *J. Hyg. Camb.* **27**, 160-273.

9. Dupont, M. C., (1931). *Le Lait.* **11**, 29-35. (Cited in Vet. Bull 1932, 2, 6).

10. Edwards, S. J. and Rippon, J. E., (1957). *J. Comp. Path.* **67**, 111-125.

11. Flaum. A., (1933). *Acta. Path. Microbiol. Scand. Suppl.* **35**, 1-137.

12. Fusillo, M. H. and Jaffurs, W. J., (1955). *J. Bact.* **70**, 481.

13. Gordon, M. H., (1903-4). *Loc. Brit. Govt. Bd. Report of Medical Office* **33**, 380. (Quoted by Flaum, 1938).

14. Gupta, S. P. and Chakravarti, R. N., (1954). *Ind. J. Med. Res.* **42**, 131-134.

15. Gwatkin, R., (1937). *Canad. Publ. Hlth. J.* **28**, 185-191.

16. Hallman, F. A., (1937). *Proc. Soc. exp. Biol. Med.* **36**, 789-794.

17. Hine, T. G. M., (1922). *Lancet*, II, 1380-1382.

18. Hucker, G. J.. (1924). *N. Y. St. Agric. Exp. Sta. Tec. Bull.* No. 100. (Quoted by Wilson and Miles, 1955).

19. Jullianelle, L. A., (1937). *Proc. Soc. Exp. Biol. Med.* **36**, 117-119.

20. Lovell, H. R., (1958). *Nature.* **181.** 995-996.

21. Minett, F. C., (1936). *J. Path. Bact.* **42.** 247-263.

22. Merchant, I. A. and Packer. R. A., (1956). *Veterinary Bacteriology and Virology, Iowa State College Press. Ames. Iowa.*

23. Nakagawa, M., (1958). *Jap. J. Vet. Res. 6,* 19-33.

24. Schlam, W. and Woods, G. H. (1953)., *Amer. J. Vet., Res.* **14**, 530-538.

25. Smith, H. W. (1947). *J. Comp. Path.* **57**, 98-115.

26. Wilson, G. W. and Miles, A. A. (1955). *Topley and Wilson's Principles of Bacteriology and Immunity.* 4th edition. Vol. I. pp. 699-725. Edward Arnold Ltd., London.

27. Winslow, C. E. A., Rothberg, W. W. and Parsons. E. I., (1920). *J. Bact.* **5**, 145-167.

STUDIES ON THE NUTRITION OF FUNGI

The influence of different vitamins on the growth of *Cercospora beticola* Sacc.

By

K. S. THIND *and* S. R. SHARMA

Botany Department, Punjab University, Amritsar

[Received on 13th November, 1959]

INTRODUCTION

Vitamin studies are useful in that fungi may be used in bioassays for the vitamin content of various products. By the proper choice of deficient organisms, it is feasible to detect minute amounts of physiologically active compounds such as the vitamins and amino acids (Lilly and Barnett), 1951). Host specificity may be found to be correlated with requirements for these factors (Wolf and Wolf, 1947).

There appears to have been made no noteworthy contribution on the nutritional requirements of this fungus. This first paper deals with the vitamin requirements of *Cercospora beticola* Sacc., causing the leaf-spot disease of spinach-beet (*Beta vulgaris* Linn. var. *bengalensis*).

MATERIAL AND METHOD

Several monosporic isolates of *C. beticola* were made from the infected leaves of spinach-beet (*Beta vulgaris* Linn. var. *bengalensis*), collected from various localities of Amritsar and Jullundur districts (Punjab). Monosporic isolate CB 7, which showed comparatively rapid rate of growth, was selected for investigating the vitamin requirements of the fungus, and is deposited in the Herbarium of the Punjab University.

The usual precautions essential in growth substance studies were observed and only analytical grade chemicals were used.

The basal medium B (Sucrose, 20 gm.; KNO_3, 5 gm.; KH_2PO_4, 2·5 gm.; $MgSO_4.7H_2O$, 0·5 gm.; $Fe_2(SO_4)_3.6H_2O$, 0·005 gm. and Distilled Water to make 1,000 ml.), devised by the authors, was found to be quite good for the growth of the fungus and was employed throughout the present work. Fifty ml. of the nutrient solution was pipetted into 250 ml. Erlenmeyer flasks.

The stock cultures were carried on solidified basal medium B. The agar used for this purpose was Difco granulated bacto- agar purified with 5% aqueous pyridine (Robbins, 1939).

As the fungus did not sporulate in culture, only the mycelial fragments were used for inoculation. The mycelium was broken down into small fragments with the help of a suitable device (fig. 1.) which consists of a petri-dish with two holes in its lid. A glass rod, with a metallic (iron) disc attached to its base, passes through the middle hole A. This hole is covered with cotton wool held in position by an

iron plate with a hole in its centre through which passes the glass rod. The second hole B is also plugged with cotton wool. After this 10 ml. of distilled water are poured into the petri-dish and the whole assembly is autoclaved. To prepare the inoculum small amount of aerial mycelium from a 9 days old culture is placed aesptcially below the metallic disc. The disc is then carefully pressed and rotated so as to thoroughly crush the mycelium below it into small fragments. The small mycelial fragments will be automatically dispersed throughout the water. One third of a millilitre of this mycelial suspension was taken aseptically with the help of a sterilised 1 ml. pipette from the second hole B and put into 50 ml. of the basal medium in each 250 ml. flask.
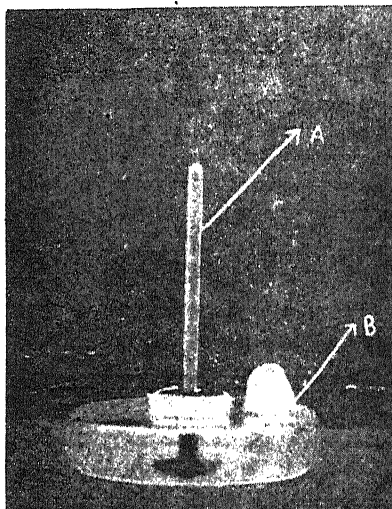

Fig. 1. A simple apparatus for the preparation of mycelial inoculation of *Cercospora beticola*.

EXPERIMENTAL WORK

Vitamin supplements were added singly or in combination in micrograms per litre of medium as follows : thiamine hydrochloride, 500μg. ; biotin, 5μg. ; pyridoxine, 500μg. ; nicotinamide, 500μg. ; Ca·pantothenate, 500μg. ; I-inositol, 40 mg. ; riboflavin, 500μg. ; para-aminobenzoic acid, 500μg. ; L-ascorbic acid, 500μg. ; choline chloride, 10 mg. ; uracil, 500μg. ; adenine sulphate, 500μg. ; xanthine, 500μg. ; guanine hydrochloride, 500μg. Stock solutions of all the 14 vitamins and growth substances were prepared before hand in distilled water and covered with a layer of toluene. All the stock solutions were stored in 5°C. refrigerator.

Initial pH of the medium was adjusted to 6 aseptically after sterilization and immediately before use by the addition of normal solutions of KOH and HCl. Sterilization was done by autoclaving at 10 lbs. pressure for 10 minutes only.

About one-third of a ml. of mycelial suspension was used to inoculate each flask. All cultures were in triplicate and incubated at 24°C for 10 days after which period the data on dry weight of the mycelium and shift in pH were

recorded. Ten days incubation period, pH 6 and 24°C temperature were found to be the optimum conditions for the growth of *C. beticola* by preliminary experiments. The complete data on vitamin nutrition which are summarized in tables 1, 2 and 3 were collected in three sets of experiments conducted under identical conditions. Data on final pH indicate that the growth of the fungus does not bring about much pH shifts. The pH is maintained in the optimum range for the growth of *C. beticola* throughout this study.

EXPERIMENTAL RESULTS

The data summarized in Table I show that growth of *C. beticola* was poor in the absence of all vitamins and growth substances as well as when only either group A or group B was present in the medium. However, the growth was very much improved when both the groups A and B were present in the medium. The data indicate that *C. beticola* shows response to the lack of certain vitamins or growth substances in both the groups A and B and that its vitamin requirements are fulfilled when both the groups are present in the medium.

TABLE I

Influence of fourteen vitamins and growth substances in different combinations on the growth of *C. beticola* after 10 days incubation at 24 'C.
Initial pH adjusted to 6.

Vitamins used	Weight of the mycelium in mg. per 50 ml. of the medium	Final pH
None	113	6·3
Group A[a]	282	6·7
Group B[b]	329	6·8
Groups A + B[c]	501	7·0

(a) Mixture of first 6 vitamins—see text.
(b) Mixture of remaining 8 vitamins and growth substance—see text.
(c) Mixture of all 14 vitamins and growth substances.

The effects of 14 vitamins and growth substances on the growth of C. beticola :

The data presented in Table 2 indicate that *C. beticola* showed poor growth when either all the vitamins and growth substances or thiamine and choline were omitted from the medium. The absence of any other single vitamin or growth substance did not result in an appreciable decrease of growth of the fungus. However, when guanine was omitted in the medium the growth was somewhat decreased although it was not as poor as when thiamine or choline was omitted. There was a slight indication that the absence of certain vitamins like riboflavin and Ca-pantothenate resulted in increased growth. However, a case of clear cut inhibition of growth in the presence of any vitamin or growth substance in the medium was never observed.

[111]

TABLE 2

Influence of omitting vitamins or growth substances singly from a combination of 14 suchsubstances present in the medium on the growth of *C. beticola* after 10 days incubation at 24°C. Initial pH adjusted to 6.

Vitamin omitted	Weight of the mycelium in mg, per 50 ml. of the medium	Final pH
All	124	6·4
None	486	7·0
Thiamine	224	6·7
Biotin	482	6·9
Pyridoxine	501	7·1
Nicotinamide	487	7·0
Ca-pantothenate	509	7·2
I-inositol	484	6·9
Riboflavin	512	7·2
Para-aminobenzoic acid	494	7·0
L-ascorbic acid	506	7·2
Choline chloride	205	6·7
Uracil	497	7·0
Adenine sulphate	492	7·0
Xanthine	498	7·1
Guanine hydrochloride	403	6·8

Influence of thiamine, choline and guanine vitamins on the growth of C. beticola:

The table 3 shows that *C. beticola* showed very poor growth when guanine alone was present in the medium. However, when thiamine or choline alone was present in the medium the growth was quite appreciable although not as good as that with all the 14 vitamins and growth substances present. The growth with combinations of thiamine and choline and thiamine, choline and guanine was equally good and similar to that with all the 14 vitamins and growth substances present in the medium.

TABLE 3

Influence of thiamine, choline and guanine, taken singly and in combination, on the growth of *C. beticola* after 10 days incubation at 24°C.

Vitamin used	Weight of the mycelium in mg. per 50 ml. of the medium	Final pH
None	117	6·4
All[a]	480	7·0
Thiamine alone	262	6·7
Choline alone	243	6·6
Guanine alone	155	6·5
Thiamine plus choline	463	6·9
Thiamine plus guanine	295	6·7
Choline plus guanine	311	6·8
Thiamine plus choline plus guanine	519	7·2

(a) All 14 vitamins and growts substances present in the medium.

DISCUSSION

This work indicates that *Cercospora beticola* Sacc. widely occurring as a leaf spot pathogen on several varieties of *Beta vulgaris* Linn. is partially deficient for both thiamine and choline. A deficiency for thiamine is by far the most common vitamin deficiency among filamentous fungi isolated from nature. Fries (1948) states that over 200 fungi are known to be partially or totally deficient for thiamine. Deficiency for this vitamin is more common among certain groups of fungi than others. Many of these fungi show only partial deficiencies, while some are totally deficient.

Only a few fungi have been reported to require choline. Thind (1950) and Lewis (1952) reported the importance of choline in procuring good growth of *Sclerotinia fructicola* and *Alternaria solani* respectively. The other two choline deficient fungi are *Glomerella cingulata*, reported by Andes (1947) and a mutant strain of *Neurospora*, reported by Horowitz and Beadle (1943).

SUMMARY

Monosporic isolates of *Cercospora beticola* Sacc. were made from the infected leaves of spinach-beet (*Beta vulgaris* Linn. var. bengalensis) and a suitable isolate CB 7 was selected for further work on vitamin nutrition.

The fungus shows partial deficiencies for both thiamine and choline.

ACKNOWLEDGMENT

*Andes, J. O., 1947. Variability of *Glomerella cingulata* from apples. Thesis for degree of Ph.D., Wisconsin Univ., U. S. A.

Fries, N., 1948. The nutrition of Fungi from the aspect of growth factor requirements. *Trans. Brit. Mycol. Soc.* 30, 118-134.

Hawker, L. E., 1936. The effect of certain necessary growth substance on the sporulation of *Melanospora destruens* and of some other fungi. *Ann. Bot.* 50, 699-717.

*Horowitz, N. H., and Beadle, G. W., 1943. A microbiological method for the determination of choline by the use of a mutant strain of *Neurospora*. *Jour. Boil. Chem.* 150, 325-333.

Leonian, L. H., and Lilly, V. G., 1942. The effects of vitamins on ten strains of *Saccharomyces eerevisiae*. *Amer. Jour. Bot.* 29, 459-464.

Lewis, R. W., 1952. The vitamin nutrition of *Alternaria solani* Phytopath. 42, 657-659.

Lilly, V. G., and Bernett, H. L., 1951. Physiology of Fungi. McGraw Hill Book Co., Inc. New York.

Robbins, W. J., 1939. Growth substances in agar. *Amer. Jour. Bot.* 26, 772-778.

——— and Ma, R., 1942. Specificity of pyridoxine for *Ceratostomella ulmi*. *Bull. Torry Bot. Club*, 69. 342-52.

———1942 b. Vitamin deficiencies of *Ceratostomella*. *Ibid.* 69, 184-203.

Thind, K. S., 1948. Variability and nutrition of *Sclerotinia fructicola* (Wint.) Rehm. Summaries of Doctoral Dissertations Univ. Wisconsin, 10 : 221-223. 1950.

Wolf, F. A. and Wolf, F. T., 1947. The Fungi, Vol. II. John Wiley & Sons, Inc. New York.

*Not consulted in original.

ROLE OF CERTAIN ANTIBIOTICS ON THE GROWTH AND REPRODUCTIVE PERFORMANCE OF SILK WORMS, *BOMBYX MORI* LINN.

By
H. C. SAXENA *and* B. K. SRIVASTAVA
Rajasthan College of Agriculture, Udaipur

Following the discovery by Stokstad and Jukes (1950) that the feeding of Aureomycin had a stimulatory effect on the growth of chicks, a number of other reports have appeared showing increased growth of chicks (McGinnis *et al.* 1950), turkeys (Saxena *et al.* 1953) and pigs (Jukes *et al.* 1950). Besides these farm animals of economic importance, antibiotics have been found to stimulate the growth of laboratory animals such as rats and mice (Vijayaraghavan *et al.* 1952) but have also been shown to be ineffective with rabbits (Lawrence and McGinnis, 1952) and even deleterious to guinea pigs (Roine and Ettalla, 1952). Recently few reports have appeared on the effect of antibiotics on the nutrition of insects. Murty *et al.* (1951) reported an increase of 6% in growth of silkworms as a result of addition of Penicillin and streptomycin to the mulberry leaves fed to the larvae. Murthy and Sreenivasaya (1953) found that chlortetracycline and chloramphenicol increased body weight and pupal weight of silk worms by 9 to 10%. Murthy *et al.* (1954) reported that larvae when treated with Chloromycetin showed increase in (*a*) rate of growth (*b*) body weight reached at maturity (*c*) weight of silk gland (*d*) quality and meterage of reelable silk (*e*) number and weight of eggs and (*f*) resistance to disease. The present communication deals with a detailed study of the role of Penicillin and a newer antibiotic Oleandomycin on certain aspects of growth and reproductive performance of silkworms. These aspects are important not only from the fundamental point of view but also from the point of view of practical sericulture.

EXPERIMENTAL

Silkworms of multivoltine pure mysore race were employed in this experiment. Six hundred freshly hatched larvae were randomly distributed into 12 groups of 50 each and reared *en masse* in seperate trays. Each treatment was offered to 4 groups which constituted a lot. Lot 1 and 2 received Penicillin and Oleandomycin respectively both at a level of 100 p.p.m. and lot 3 served as a control. *En masse* rearing was practised and 20 grams of fresh, chopped mulberry leaves were fed to each group five times a day during the early stages. After the third moult silkworms were fed 40 grams of leaves seven times a day. The required quantity of antibiotics were incorporated in the diet by dusting the premix over the thinly spread chopped leaves and then mixing it thoroughly by hand in enamelled cups. The premix was prepared in lactose in convenient amounts for efficient dusting. The control groups received an equal amount of lactose only.

Biological data on moulting, growth, spinning and emergence were regularly recorded. The larvae were weighed individually on the 5th day after the fourth moult, while the weight of green cocoons was taken 3 days after spinning was

[115]

over. The moths were mated soon after emergence and eggs were allowed to be laid on a sheet of filter paper which were ccunted socn after laying was over. The eggs were then maintained at room temperature (25-27°C) until they hatcked. The 'ants' obtained from each of the 'layings' were counted and the percentage of hatchability in each case determined. Data on the size of adult moths and the length of incubation period were also recorded.

In order to compare the overal development, growth index was caculated by the method employed by Singh and Pant (1955) in their insect nutritional studies. The Index was obtained by dividing the percentage of larvae that started spinning (N) by the average time taken to complete the development (Av). The greater the value of index the better was the development of silkworms.

RESULTS AND DISCUSSION

Result of the study are presented in Table 1 and 2. It is evident from the data presented in Table 1 that both the antibiotics produced significantly higher

TABLE 1

Effect of antibiotics on growth and reproductive performance of silkworms

| Lot No.	Supplement.	Av. Wt. of Larvae in fifth instar gm.	S. E.	Percentage Growths Response	Moult period fourth, Hrs.	Av. Wt. of Green Cocoons gm.	No. eggs laid per laying.	Hatchability %
1	Penicillin	0·7571	0·0143	33·6	30*	0·6517	394	92·4
2	Oleandomy-cin	0·7233	0·0031	27·7	25	0·6866	425	93·6
3	None	0·5664	0·0969	..	36	0·6250	265	83·1

*Measured when 75% of the larvae were out of moult.

TABLE 2

Effect of antibiotics on the development of silkworms

Lot No.	Supplement	Percent larve started spinning (N)	Average time taken for complete development in days.		Growth Index N/Av
			Range	Average (Av)	
1	Penicillin	67·5	32—34	32·3	2·09
2	Oleandomycin	90·0	32—34	32·1	2·80
3	None	29·0	33—35	34·0	0·86

larval weights at maturity. The larvae receiving Penicillin and Oleandomycin weighed on an average 0·7571 gm. and 0·7233 gm. respectively, while the worms from the control group averaged 0·5664 gm. only. The growth responses of the silkworms to Penicillin and Oleandomycin were 33·6 and 27·7 per cent respectively. The difference in wieght between Penicillin and Oleandomycin groups was not significant. A great deal of variation (Fig. 1) was noticed in the size of the larvae of the control groups. The variation was comparatively less in penicillin groups and almost negligible in case of larvae receiving Oleandomycin. These observation were found true statistically as is evident from the S. E. values shown in Table 1.
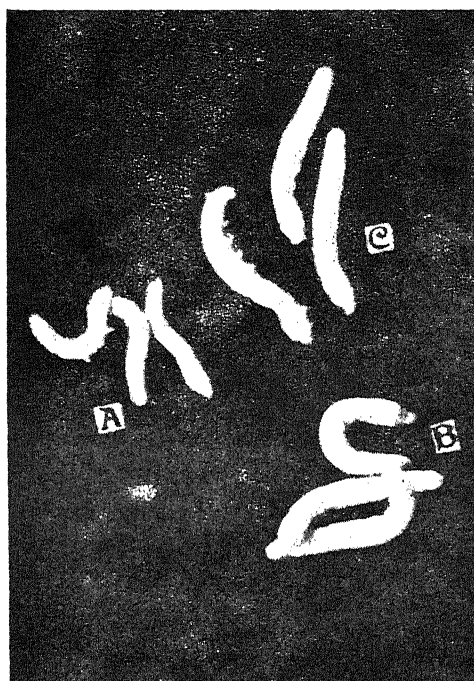
Role of certain antibiotics on the growth and reproductive performance of silkworms, *Bombyx mori* Linn.

The antibiotics shortened the duration of the different moults which was however significant only in the 4th moult. Oleandomcyin proved better in reducing the moulting period as compared to Penicillin. It took 36 hours for moult in case of worms of control groups as compared to 30 hours for Penicillin groups and 25 hours for Oleandomycin groups.

The weight of green cocoons were also found enhanced due to antibiotic supplementation; Oleandomycin producing greater gains than Penicillin in all groups. Cocoons from worms receiving Oleandomycin weighed on an average 0·6866 gm. as compared to 0·6517 gm. for Penicillin and 0·6250 gm. for control groups.

The addition of the antibiotics to the diet of the larve resulted in increased fecundity and hatchability. On average a female moth laid 265 eggs in control

groups as compared to 394 in Penicillin and 425 in Oleandomycin groups. The hatchability figures were 83·1% for control groups, 92·4% for Penicillin groups and 93·6% for Oleandomycin groups. There was no difference in the size of the adults produced and the incubation period of the eggs.

The data presented in Table 2 shows that the antibiotic supplementation greatly reduced the mortality of the larvae. Oleandomycin was shown to have a better effect than Penicillin. Overal development as judged by the Growth Index figures was definitely in favour of Oleandomycin (2·80), though Penicillin (2·09) was also found to be very much superior than the conrol groups (0·86).

The results obtained in this study showed that the newer antibiotic Oleando-mycin proved more effective in increasing the growth and reproductive performance of silkworms. Saxena and Kumar, (1959) in their work with chicks reported a greater response with Oleandomycin as compared to Terramycin and Penicillin. The method developed at this Laboratory for feeding antibiotics to the worms was considered more efficient and superior to the one employed by Murthy et al. (1954) in which worms were fed by smearing the mulberry leaves with measured amounts of a standard solution of the drug. The method used in the study under report was found to be more practical, less cumbersome and maintained accurate level of the drug fed to the silkworms.

In our study the worms were reared en masse in batches of 50 in enameled trays which was more comparable to the conditions of rearing in sericultural practice. Each treatment was offered to 4 such groups which provided an excellent experimental design for testing the results statistically. Penicillin was included in this study to compare the performance of the new antibiotic Oleandomycin. The results obtained with Penicillin are in agreement with the results reported by Murthy et. al. (1951) except that the response obtained in our study was of much high order than reported by them.

SUMMARY

Role of certain antibiotics on the growth and reproductive performance of silkworms, Bombyx mori, Winn. has been studied. Larvae when fed Penicillin, and Oleandomycin showed increase in (a) rate of growth (b) body weight reached at maturity (c) number of eggs laid (d) hatchability (e) weight of green cocoons and resulted in a better overall development of worms as shown by an increased Growth Index.

The antibiotic supplementation reduced (a) mortality of the larvae (b) varia-tion in the size of the larvae and (c) the period of moulting which was significant in the 4th moult. It however did not produce any effect on the size of the moths and the incubation period of eggs.

An improved method of feeding antibiotics to the worms has been des-cribed.

REFERENCES

Jukes, T. H., Stokstad, E. L. R., Taylor, R. R., Cunha, T. J., Edwards, H. M., and Meadows, G. B. (1950) *Arch. Biochem.* 29 : 452.

Lawrence, J. M. and McGinnis, J. (1952) *Arch. Biochem.* 37 : 164.

McGinnis, J., Berg, L. R., Stern, J. R., Wilcox, R. A., and Bearse, G. E. (1950) *Poultry Sc.* 29 : 771.

Murthy, M. R. V., Shankarappa, B. S. and Sreenivasaya, M. (1951) *Curr. Sci.* 20 : 269.

Murthy, M. R. V. and Shreenivasaya, M. (1953) *Nature* 172 : 684.

Murthy, M. R. V., Shankernarayana, B. and Shreenivasaya, M. (1954) *J. Sc. Ind. Res.* 13B : 331.

Roine, P. and Ettalla, T. (1952) *Nature* 169 : 1014.

Saxena, H. C., Blaylock, L. G., Stadelman, W. J., Carver, J. S. ard McGinnis J. (1953) *Poultry Sc.* 32 : 721.

Saxena, H. C. and Kumar, K. (1959) *Ind. Vet. J.*, 36(12) : 575.

Singh, K. R. P. and Pant, N. C. (1955) *J. Zool. Soc. India* 7(2) : 156.

Stokstad, E. L. R. and Jukes, T. H. (1950) *Proc. Soc. Exper. Biol. and Med.* 73 : 523.

Vijayaraghavan, P. K., Murphy, E. A. and Dunn, M. S. (1952) *Arch. Biochem.* 36 : 127.

STUDIES ON FURCOCERCOUS LARVAL TREMATODES FROM INDIA

Part—I. A Critical Review Interrelationships and Taxonomy

By

R. N. SINGH*

College of Science, Raipur, M. P.

[Received on 6th June, 1959]

INTRODUCTION

The study of larval forms of digenetic trematodes has its own significance. The structure, behaviour and ecology of trematodes along with an increased knowledge of larval forms, their intermediate and final hosts have always been useful in solving the life history problems. During the past few decades the importance of the various larval stages and their development has been greatly realized as such studies have provided sufficient evidence of phylogenetic relationships among various groups of trematodes. Further, such studies have been of great significance as they have led to the emergence of system of classification of digenea based on evolutionary history of the group.

The notable contributions made by Luhe (1909), Lebour (1912), Cort (1917), Leiper (1915) on larval forms gave impetus to other workers in the field. Soparkar (1921), Sewell (1922), Faust (1917-1924), Brown (1926), Dubois (1929), Miller 1923, 1926 and 1927) and Miller E. L. (1936) have made valuable contributions to the study of larval forms and life cycles of digenetic trematodes. The name of Brown, Ciurea, Cole, Dollfus, Ejsmont, Harper, Hughes, Hunters, Krull, La Rue, Lutz, Mathias, Porter, Price, Ruzskowaski, Stunkard, Szidat, Wesenberg Lund and others also stand out prominently for their contributions on larval forms and life cycles of digenea.

A survey of literature in India reveals that valuable work on larval forms led by Sewell (1922) has been followed by a number of workers and has produced promising results. The present work has been undertaken with a view to publish a consolidated account of all furcocercous cercariae discovered from India so far and to give a brief description, systematic position and relationship of each form in view of recent works on the life history of trematodes having forked-tailed cercariae as their larvae.

A CRITICAL REVIEW

The study of larval stages began in the 2nd half of the 18th century, when the names "Cercaria" of O. F. Muller (1773), "Furcocerca" of Lamarck (1815) and "Histrionella" of Bory de St. Vincent (1823) were used as generic names for supposedly adult worms. Bojanus (1818) described cercariae and observed that they were produced in yellow sacs within snails. He called these yellow sacs as

---

*Present Address : Head of Deptt. of Zoology, Mahakoshal Mahavidyalaya, Jabalpur (M. P.)

[120]

'royal yellow worm'. de Filippi (1837) assigned the generic name "Redia" for these yellow worms and in 1854 he stated that they were larval stages and not the adult flukes. As such, he advocated that name "Redia" should not be used to designate a genus.

The relation of cercariae to adult worms was for the first time indicated by Oken in a foot note to the papers of Nitsch (1817) and Bojanus (1818). He stated that cercariae are embryos of distomes.

The ciliated larva (embryo) named miracidium by Braun (1892) was observed by Mehlis (1831) in the egg of *Monostomum flavum* and by v. Siebold (1835) in the egg of *M. mutabile.* v. Siebold also observed that miracidium of *M. mutabile* contained a larva with pharynx, simple gut and thus established the fact that redia is the offspring of a miracidium. The source of the term "Sporocystis (sporocyst) is not known though it was used by de Filippi (1854) and Stiles and Hassall (1908) for the first time. The accumulation of observations made by above workers concerning the life history of trematodes led Steenstrup (1842) to consider metagenesis (alternation of generation) as a process in the reproduction of coelenterates, trematodes and other lower organisms. His observations were supported by v. Siebold (1850) and several others. Although well before Steenstrup it had already been etablished that cercariae were larval stages, interest in the study of these forms did not cease and several works appeared specially by de Filippi, La Valette St. George and others. Diesing (1850) however, still considered cercariae to be adult worms and created a suborder for them. In 1855 he recognized 9 genera and 20 species of cercariae. However, in 1850, he accepted the view that cercariae were larvae but continued to use generic names for them.

Inspite of many attempts to determine the life histories of trematodes during the period 1843-1892, the first life history was not discovered until 1882 when Leuckart and Thomas (1883) independently worked out in detail the life history of *Fasciola hepatica,* the common liver fluke of the sheep.

Sonsino (1892) described larval forms outside Europe and Looss (1896) described furcocercous cercariae in Germany and neighbouring countries. After Diesing, Luhe (1909) made the first attempt to classify cercariae in a comprehensive manner. He recognized five major groups : Lophocercariae, Gasterostome cercariae, Monostöme cercariae, Amphistome cercariae and Distome cercariae. He based his observations on morphological grounds and made use of the characters such as the presence or absence and locations of suckers, presence and absence of stylet, form of the tail and other structures. He further, subdivided the distome cercariae into Cystocercous, Trichocercous, Furcocercous, Microcercous and Gymnophalus cercariae as well as tailless cercariae. After Luhe, numerous attempts have been made to modify his scheme and improve the conception of relationships between the various types of cercariae but still the scheme forms the basis of any alternative one puts forward at the present time. However, Cable (1956) has vigorously questioned the further usefulness of Luhe's scheme of classification in the present state of knowledge of larval forms and their life histories.

Sinitsin (1911) studied cercariae and to some extent their development. He compared the development of the peculiar Bucephalid larva with that of the forktailed *cercaria ocellata* whose adult is now known to be a blood fluke (*Trichobilharzia ocellata*). However, except in a few instances he seems to have made no particular reference to account for the relationships of cercarial types or to families of adult flukes (worms).

[121]

Lebour (1912) presented an alternative scheme for the larvae and used the development in sporocysts or rediae of these larval forms as a basic factor in her scheme of classification in addition to the taxonomic importance of the tail. This scheme, though could not be widened to include all the known forms, yet it attempted to lay more emphasis on the mode of origin of cercariae. She drew more attention to the separation of forms which in other respects show close resemblance due to loss of tail by abbreviation of the life cycle.

Leiper (1915) described furcocercous cercariae in much detail and separated the schistosome cercariae from other group of furcocercous forms on account of the lack of a pharynx, of pigmented eye spots, cuticular keel on the furcae, length of the furcae which are less than half the length of the tail stem.

Cort (1917) suggested a scheme on the basis of his study of five furcocercous cercariae and laid special emphasis on the excretory system as the important criteria in classification. He writes "A mere complete knowledge of this system will do much to clear up relationship and to establish natural families. Also an increased knowledge of the excretory system of little known types of cercariae will be of great help in solving life histories by suggesting the groups of adults to which such forms belong." He thus concluded that five cercarial species were related, the three without pharynges and the two having pharynges represent at least two distinct families. For the apharyngeate species, C. douthitti and C. elephantis he suggested a relationship within a family. Later work has proved both these forms to be blood flukes the first in the family schistosomatidae and the second in the family spirorchiidae. The close relationship of these two families is fully established. C. douglasi and C. emarginatae, the two pharyngeate species are now known to belong to the Strigeatoidea. Cort thus concluded the conservatism of the excretory system and its value in establishment of relationships in trematodes.

Faust (1917) originally came to the conclusion that all fork-tailed distome cercariae were members of the schistosomidae. This group of larval trematodes he characterised as being fork-tailed and without a pharynx. Subsequent discoveries of other forms which did not fall within the limits of Faust's Schistosome larval groups led Faust (1918) to modify his views and to enlarge the boundaries of his schistosomid group. He writes "with the broadening knowledge of schistosome larvae it seems more reasonable to recognize a complete series of larval forms those with a pharynx sphincter (C. douglasi, C. emarginatae, and perhaps C. vivax Sonsino) through those with a degenerate pharynx with or without intestinal caeca (C. gracillima, C. minor) through those without any pharynx but with well developed mucin glands (C. gigas, C. tuberistoma, C. douthitti) to the human schistosome cercariae." Faust paid too much attention to the presence or absence of pharynx and intestine and did not sufficiently take up into account the presence and absence of other anatomical characters of equal importance.

Sewell (1922) modified the scheme of Luhe in many respects though not satisfying subsequent workers in the field. He made use of such characters to which Luhe attached much importance. He extended such characters and formed many groups of cercariae where Luhe for want of information could not do so and contented with solitary examples but with characteristic features. Sewell attempted to derive the distomes from monostome stem and recognized six groups of monostome cercariae : Pleurolophocerca, Ephemera and Urbanensis groups which develop in rediae and the Ubiquita, Lophocerca and Lophoides groups which develop in sporocysts. He believed each of these groups to be a starting point for

a series of lines of evolution each of which gives rise to distome descendants. Sewell modified the classification of distome furcocercous cercariae as given by Cort (1917) in the point that he combined Cort's group I and II into one whereas he divided group III of Cort into two groups. Group I of Sewell includes the brevifurcate, apharyngeal forms divided into two series mainly depending on the presence or absence of hollow spines capping the ducts of the penetration glands. Group II includes the Longifurcate cercariae divided into two series depending on the presence or absence of pharynx. Group III is meant to contain some peculiar forms with leaf like appearance. However, Sewell's ideas incorporated in his monographic work on Indian Cercariae proved untenable in the light of recent works specially of Stunkard (1934) who concluded that both distomes and monostomes are polyphyletic groups and that neither can be regarded as simple primitive type. He further advanced the thesis that certain distomes appear to have derived from monostomes whereas certain monostomes appear to be derived from distome ancestors.

Faust again in 1924 divided furcocercous forms according to the excretory systems flame cells formula because he believed that it was a natural basis for natural grouping. But his belief that the excretory system is a common system carried over from the cercaria to the adult was refuted by Miller's (1926) statement that the flame cells of the cercariae might have potentialities for more rapid division so that the pattern of the adult worm could not be predicted. This has been illustrated in other form for instance in the larva and adult of *S. japonicum*. However, Faust included the monostomes and distomes furcocercous cercariae into one homogeneous group and laid much stress on the excretory system to establish natural groupings. Stunkard (1929) advocated caution in the use of the excretory system for natural groupings of these larvae because in most trematodes the development of the cercaria to the metacercaria and the adult is accompanied by increase in the number of excretory ducts and flame cells. Brown (1933) showed in *Lecithodendrium chilostomum* the same type of excretory system serving all stages of development and he suggested that an increase in the number of ducts during development might be an expression of the physiological needs of the organism and similarly in the number of excretory units in a group or of groups within the system, the result of convergence in evolution and not necessarily an indication of phylogenetic relationship.

La Rue (1926) extended Cort's idea to include relationships between certain superfamilies and orders of the digenea. He proposed that the number of flame cells in the miracidia might give additional support in some cases to the claims of relationships. On the basis of his analysis of the results of the life history work of Cort, Sewell, Faust and others on one hand and that of Lutz, Ruszkowski and Szidat on the other, he proposed the order Strigeatoidea with three suborders : Strigeata, Schistosomata and Bucephalata. Again in 1938 he reviewed the literature pertaining to the excretory system as a basis for a natural classification of the Digenea.

The most recent system of Classification of these cercariae is by Miller (1926). He attached great importance to the presence or absence of a pharynx and stated : "The presence or absence of a pharynx is the first consideration of the division of the furcocercous larvae into two main groups." He supported his view by Stunkard (1923) on the adult blood inhabiting trematodes from the reptiles and their relationships with the forms found in fishes, birds and mammals. He further wrote : "These studies indicate that the blood flukes constitute a natural group.

[123

They on the other hand lack a muscular pharynx and so it seems that the presence or absence of a pharynx is of great significance and the possession or lack of a ventral sucker is relatively unimportant." Miller divides these two main groups Pharyngeal and Apharyngeal into two subgroups Brevifurcate and Longifurcate. Each of these he further divides into Monostome and Distome groups, except the pharyngeal brevifurcates in which no monostome cercaria has been recorded so far.

Although Miller's 1926 classification of furcocercous cercariae has been accepted to day by a large number of workers yet a good deal of misunderstanding and confusion may lead to wrong allocation of cercariae in view of recent findings. From data on the life history of various groups of trematodes in the past three decades, it has become more or less clear that the value of different characters for the division of furcocercous cercariae into distomes or monostomes, longifurcate or brevifurcate, pharyngeal or apharyngeal and their development in rediae or sporocysts have relatively grown very narrow and requires reorientation. The most illustrative and glaring examples leading to this contention are schistosome and strigeid cercariae and cercariae belonging to the families Aporocotylidae, Sanguinicolidae, Spirorchiidae, Brachylaemidae and Clinostomatidae all included under the order Strigeatoidea La Rue, 1926.

As already pointed out Miller (1926) while recognizing the presence or absence of a pharynx as a character of greater importance than the presence or absence of an acetabulum in furcocercous cercariae also laid emphasis on the character of the tail. He divided both pharyngeal and apharyngeal furcocercous cercariae further into longifurcate and brevifurcate subgroups and assigned special distinctive features to each subgroup. The terms longifurcate and brevifurcate were mainly used to denote the length of furcae in relation to the tailstem length. A cercaria having furcae without constrictions at the base and longer than one half the length of the tailstem length or exceeding that is called longifurcate whereas the term brevifurcate is used when the furcae are less than half the length of the tailstem and are constricted off from the base. In brevifurcate forms the tail is usually narrow than the body and in longifurcate forms its width is generally equal to that of the body. However, the tailstem and furcae are extremely contractile structures and it is doubtful if the length of the two structures could be adequately appraised in all cases. Since long, probably an impression has been formed that schistosome cercariae are brevifurcate and Strigeid longifurcate but in schistosome cercariae themselves the fundamental character of a brevifurcate form is not perfectly established as the following examples show. Miller (1926) himself has erred by putting C. elvae [= C. ocellata according to Dubois (1929) and Taylor and Baylis (1930)] with caudal furcae and tailstem as 238μ and 501 (living material) and 290μ and 382μ (balsam preparation) and cercaria gigas with caudal furcae and tailstem as 320μ and 180 . Porter (1938) gives the measurement of caudal furcae and tailstem in the cercaria of Bilharziella polonica as 169μ and 2 0μ. In C. ocellata which Brumpt (1931) found to be the cercaria of Trichobilharzia ocellata as reported by Porter (1938), the caudal furcae and tailstem measure 230—310μ and 370 - 430uμ. These two schistosome cercariae are, therefore, longifurcate. Talbott (1936) has given the lengths of caudal furcae in relation to the tailstem of four schistosome cercariae and in all these furcae are longer than half the length of the tailstem and so it is difficult to put these cercariae in the brevifurcate group. Macfarlane and Macy (1946)[1] have described long caudal furcae in the schistosome cercaria, C. oregonensis. The tailstem and furcae are nearly equal in C. biocellata Singh, 1955. The above examples thus do not establish the fundamental value of longifurcate and brevifurcate characters.

[124   ]

Of the other characters assigned by Miller (1926) to differentiate between brevifurcate and longifurcate forms, a majority are more or less common to both the groups. In the nature of spination, presence or absence of long hairs on body, or tail, nature of gut, eye spots, head glands, penetration glands, flame cell number in tail and furcal finfolds, there does not exist a very sharp difference between the two sub-groups as is clear from the morphology of Aporocotylid-Sanguinicolid, Spirorchid, Schistosome and Clinostome cercariae which are brevifurcate and Strigeid which are longifurcate as well as the more specialized cercariae of the families Bucephalidae, Brachylaemidae and Fellodistomatidae. In fact, the nature of the tail and tail-furcae have become modified in various groups of the Strigeatoidea according to mode of life.

From the above explanation it is more or less evident that the basis of differentiation into brevifurcate and longifurcate cercariae is not in confirmity with the available data. The sharply restricted grouping based on the character of the tail and furcae has been proved to be rather arbitrary. As such our conception of a longifurcate or a brevifurcate nature of cercaria in relation to Schistosomes, Strigeids and other related forms under the Strigeatoidea requires change. There-fore, it appears safe to retain the terms brevifurcate and longifurcate for conve-nience of description rather than using them as designations for the two sub-groups.

Similarly if we consider the nature of the pharynx in furcocercous cercariae, we find that the structure is of variable nature. Its absence characterises the entire group of blood-flukes but Oiso (1927) has shown a muscular pharynx in the ceraria of *Bilharziella yokogwai* which is lost in the adult. *Cercaria gracillima, C. minor,* and several strigeid cercariae also possess degenerate pharynx. Cercaria of *Clinostomum marginatum* has a delicate pharynx which is lost in the adult but present in the metacercaria. In other Clinostome cercariae that are known, a pharynx is not present. The cercaria of *Apharyngostrigea pipientis* (Faust) has a pharyngeal bulb but the adults lack the structure. *Cercaria multicellulata* wrongly placed in the pharyngeal group by Miller actually does not possess a pharynx. These observa-tions show that the pharyngeal or apharyngeal condition of longifurcate or brevifur-cate forms is a transitory character and has become modified according to the mode of development and possibly physiological adjustment in the hosts. The presence or absence of a ventral sucker is also of no prime importance as already established by Stunkard (1934) and others. It is well known that among blood flukes many are distomate, some monostomate and certain strigeids have also no acetabulum. As such the division of cercariae into pharyngeal and apharyngeal, distome or monostome does not appear to be sound.

Following the lead of Sewell (1922) and others, Dubois (1929) based classi-fication of cercariae mainly on the basis of excretory system in addition to other characters. He recognized in his group of monostome cercariae two types of excretory system which he gave the names "Stenostoma" and "Mesostoma". In the former type the lateral excretory canals extend forward to the pharynx and turn about dividing into anterior and posterior branches. In the latter type, the excretory canals extend only to the vicinity of the ventral sucker there dividing into anterior and posterior vessels. He also showed other characteristics to be corelated to the two types of excretory systems e. g. cercariae with Stenostoma Type of excretory system develop in rediae and those with Mesostoma Type deve-lop in sporocysts and the miracidia have two pairs and one pair of flame cells respectively. Xiphidiocercariae and furcocercous cercariae show the mesostoma

[125]

type whereas Amphistomes, Echinostomes and Gymnophalus carcariae show the Stenostoma type. From such correlations, Dubois inferred that some digenea followed one or another course in evolution. Other groups of cercariae showing combinations of characters in one or the other series are Lophocercariae (San guinicolidae) Cystocercous (*Gorgodera cygnoides* : Gorgoderidae), Microcercous (*Sphaerostoma bramae* : Allocreadiidae), Rhopalocercous cercariae of the Ispori and Pleurolophocerca groups, cercariae of the Helicis and Leucochloridium group and perhaps the monostome cercariae belonging to the Notocotylidae. Though the group "Cercariaea" form an unnatural one, however, many of them have the characters of the Stenostoma Type. Except lophocercariae many others which have been studied seem to have followed one guggested line of evolution or the other. But an examination of literature concerning the life history of digenea shows that relationships do not exist as both mesostomate and stenostomate types of excretory systems occur in the cercariae of the Superfamily Opisthorchoidea as well as within the single subfamily like Gorgoderinae.

La Rue (1938) while reviewing the literature concerning the importance of excretory system as a basis for a natural system of classification of digenea expressed the view that : "Much more information is needed on the process of formation of the excretory vesicle. With large mass of data on this process it might be possible to determine whether it is of significance in the development of a natural system of classification." He further said, "the development of a natural taxonomic system for the digenea must be based upon comparative anatomy of all stages in the life history. Account must in any case be taken of the miracidium, especially of its flame cells and possibly of the number and arrangement of the epidermal plates. In the cercaria attention must be given first to form or type of cercaria. Type of cercaria usually takes penetration apparatus into account as well as tail and other external characters . . . . Of special importance in the cercaria are the pharynx, and the character of the excretory system including flame cell pattern, forms and proportions of excretory vesicle. Possibly the formation of the excretory vesicle and the excretory pore may have value as characters".

Having above ideas in view, La Rue (1957) has given a system of classification for Digenea based largely on similarities in the life cycles and developmental stages and of the excretory system which has been discussed in the coming pages.

CERCARIAE AND THE TAXONOMY OF THE DIGENEA

The modern concept of classification is to portray genetic relations by taxonomic symbols (Stunkard, 1946). The trematode classification emerging to day is largely based on phylogenetic relationships. Data for determinations of phylogeny in various groups of trematodes have been derived from comparative morphology and life history as well as host parasite relations and physiology. These studies which are in a way very recent have changed our views about the older taxonomy of the digenea, presented by earlier workers like Van Beneden (1858) Monticelli (1892), Braun (1893), Looss (1899), Odhner (1905, 1912), Poche (1926), Fuhrmann (1928) and others.

La Rue (1926) for the first time laid a key note based on life histories, development and corelation between the adult and larval stages for a revision of the classification of the digenea. He created the order Strigeatoidea under the digenea to include three major groups : blood-flukes, strigeids and gasterostomes. This led to the creation of several orders and superfamilies based on life history studies

by subsequent workers.   Thus were added the Clinostomatidae by Hunters (1934), Azygiidae by Szidat (1932), Brachlaemidae by Allison (1943) and Fellodistomatidae by Cable (1953) to the Strigeatoidea.   Allison (1943) and Mehra (1950) created the suborders Clinostomata and Brachylaemata respectively under the Strigeatoidea.

Stunkard (1946) laid emphasis that "no deep significance can be attached to the presence, location or absence of an acetabulum and the arrangement of these worms into the suborders Amphistomata, Distomata and Monostomata has little phylogenetic or taxonomic support."   To support this argument, he further stated, "the time factor is important, an acetabulum may appear in the cercaria and continue its development in the distomes, it may appear in the cercaria and fail to develop further in certain Cyclocoelids and Eucotylids ; it may not appear till later for example, in the metacercarial stage of certain microphallids, heterophyids, and opisthorchids or it may fail to develop at all in the so called monostomes."   He therefore, proposed to suppress the orders Gasterostomata and Prosostomata and in the latter the suborders Amphistomata, Distomata, Monostomata, Strigeata, Bucephalata, Schistosomata and Clinostomata.

La Rue (1957) has again presented a new system of classification of the digenea based exclusively on the method of development of excretory system, similarities in the life-cycles and development of the larval-stages.   In this system he has dropped the *taxa* Gasterostomata Odhner (1905) and the Prosostomata Odhner (1905) as these *taxa* were based on the position of the mouth, a character of no great significance.   However, he has preserved the value of the adjectives gasterostomate and prosostomate in characterising cercariae and adults and prefers their use in a purely descriptive and not in a taxonomic sense.   La Rue divides the digenea into two Super orders : Epitheliocystidia and Anepitheliocystidia.   In the former, the excretory bladder in cercarial, metacercarial and adult stages is composed of epithelical cells which are derived from the mesoderm and cercariae are with tails sinple, reduced in size or lacking, caudal excretory vessels present or absent, stylet present or absent, miracidium with one pair of flame cells. In the latter superorder, the excretory bladder is thin walled and not epithelial at any stage, caudal excretory vessels present in developing cercariae, stylet absent, miracidia with one or two pairs of flame cells, or apparently lacking.

The super order Anepitheliocystidia La Rue, 1957 includes three orders : Strigeatoidea La Rue, 1926 ; Echinostomida La Rue, 1957 and Renicolida La Rue, 1957.   The order Strigeatoidea contains four suborders : (1) Strigeata La Rue, 1926 with three superfamilies; Strigeoidea Railliet, 1919 ; Clinostomatoidea Dollfus, 1931 and Schistosomatoidea Stiles and Hassal, 1926 ; (2) the suborder Azygiata La Rue, 1957 with the superfamily Azygioidea Skrjabin and Guschanskaja, 1956 and Transversotrematoidea La Rue, 1957 ; (3) the suborder Cyclocoelata La Rue, 1957 with the superfamily Cyclocoeloidea Nicoll, 1934 and (4) the suborder Brachylaimata La Rue, 1957 with three super families : Brachylaimoidea Allison, 1943, Fellodistomatoidea La Rue, 1957 and Bucephaloidea La Rue, 1926.

The order Echinostomida La Rue, 1957 includes two suborders ; Echinostomata Szidat, 1939 with the superfamily Echinostomatoidea Faust, 1929 ; the Paramphistomata Szidat, 1936 with the superfamily Paramphistomatoidea Stiles and Goldberger, 1910 and Notocotyloidea La Rue, 1957.

On the basis of life history data provided by Cable (1954a), La Rue includes the family Haplosplanchnidae Poche, 1926 in the Echinostomatoidea although fishes are the final hosts of the adults.   Further, according to him the morphology

] 127]

of the cercaria and of the adults except for the lack of the collar and collar spines is in accord with the characters of the superfamily. He includes the family Heronimidae in the Paramphistomatoidea because of the amphistomate cercaria in the life history of *Heronimus chelidrae* according to Cable and Crandall (1956).

The order Renicolida La Rue, 1957 with the single suborder Renicolata La Rue, 1957 and with the single superfamily Renicoloidea La Rue, 1957 is included in the Anepitheliocystidia on the basis of life cycle data provided by Rothschild (1935), Wright (1956) and Cable (1956).

The order Strigeatoidea La Rue, 1926 includes all families of digenea which have forked-tailed cercariae. La Rue includes the Clyclocoeloidea in the Strigeatoidea due to cercariae having a bilobed condition of the much reduced tail as described and figured by Timon-David (1955) for the cercaria of *Pseudhyptiasmus dollfusi*. He states that "the lack of branching sporocysts and the presence of a well developed redia in the life cycle rule out the possibility that the Cyclocoelids are closely related to the Branchylaimidae."

While discussing the position of the family Fellodistomatidae near the Brachylaimoidea in the order Strigeatoidea, La Rue supports his arguments by the studies of Cable (1953-1954b, 1954c and 1956). Cable showed that Gymnophalline (Fellodistomatid) cercariae are forked-tailed, simple tailed or without tails and the morphology of their body demonstrates relationship within the subfamily.

The superorder Epitheliocystidia includes two orders : Plagiorchiida La Rue 1957 and Opisthorchiida La Rue, 1957. The Plagiorchiida contains the singll suborder Plagiorchiata La Rue, 1957 with two superfamilies : Plagiorchoidea Dolfus, 1930 and Allocreadioidea Nicoll, 1934. The order Opisthorchoida has two suborders : Opisthorchiata La Rue, 1957 with the superfamily Opisthorchioidea Faust, 1929 and the suborder Hemiurata Skrjabin and Guschanskaja, 1954 with the Superfamily Hemiuroidea Faust, 1929. La Rue has provisionally placed the family Didymozoidae under Hemiuroidea on account of the similarity of excretory systems in the adults and the miracidia as shown by Cable (1956).

The superfamily Allocreadioidea Nicoll, 1934 according to La Rue contains a variety of cercarial forms and, therefore, it is not a natural one and requires subdivision into several subfamilies more or less closely related and in the present state of knowledge its revision is out of scope. The families Gyliauchenidae and Opistholebatidae have been provisionally included in the Superfamily Allocreadioidea on the basis of comparative morphology. The position of the family Troglotrematidae is problematical as it has a life cycle resembling those of Allocreadioidea and the Plagiorchioidea. However, on the characters of the uterus and the large size of eggs the family Troglotrematidae has been placed within the Allocreadioidea.

Mehra (1957) in his classification of digenetic trematodes considers that the Superorders Anepitheliocystidia and Epitheliocystidia are unnecessary and should be dropped as the development of the epithelial bladder in the cercariae of the latter superorder is considered to be a secondary character having no phylogenetic significance and showing no clear relationship of certain groups included under it to one another than to some groups of the former superorder. For instance, he considers that suborders Echinostomata and Paramphistomata of the Anepitheliocystidia are more closely related to the Plagiorchiata and Opisthorchiata than to the suborder Strigeata La Rue, 1926 and Brachylaemeta Mehra, 1950. Further,

the Suborder Cyclocaelata is more closely related to the Paramphistomata and Notocoyloidea of the order Fasciolatoidea Szidat, 1936 than to the order Strigeatoidea La Rue, 1926 and thus is also closely related to the order Plagiorchiida La Rue, 1957. The loss of great reduction of tail in the cercariae of these groups of trematodes has apparently taken place separately by convergence in some groups as an adaptation to a life cycle in which the free swimming stage is omitted as already pointed out by Cort (1918) and Wallace (1941). On these points, Mehra does not consider Cyclocaelata to be closely related to Brachylaemata Mehra, 1950 and hence to the Strigeatoidea La Rue, 1926.

Further in his opinion, a comparision of the development of the excretory system from the embryo to the mature cercariae shows that the entire excretory system minus the development of the definitive excretory bladder in mature cercariae should constitute an important basis of classification with some other features of the developmental stages and the adults and this invalidates the super orders created by La Rue. Thus in his scheme, he drops the order Echinostomida La Rue, 1957 and replaces it with Fasciolatoidea Szidat, 1936 on the basis of priority and accepts the orders Plagiorchiida La Rue, 1957 and Opisthorchiida La Rue, 1957. Further, he creates two new orders Azygiatoidea and Hemiuratoidea. The former includes the suborder Azygiata with the superfamily Azygioidea Skrjabin and Guschanskaja, 1956 characterised by having gigantic furcocystocercous cercariae with flame cells groups in the tail, development in sausage shaped radiae with terminal rudimentary pharynx but without gut and locomotary appendages, genital opening in front of the oral sucker, miracidia probably nonciliated. The Hemiuratoidea includes the suborder Hemiurata Skrjabin and Guschanskaja and is characterised by having Cystophorous or Hemiurid cercariae with primitive type of development of the excretory system in which the fused tubes extend upto the tips of one part of the highly modified tail, cercariae develop in rediae, second intermediate host a copepod, miracidia non ciliated.

In another paper, Mehra (1958) has discussed the relationships of the family Gorgoderidae with the families Azygiidae and Hemiuridae. He has shown that the cystocercous or cyst forming character of the tail of Macrocercariae and Rohpalocercariae of the Gorgoderidae is of a deep seated nature so as to be considered of phylogenetic importance indicating the genetic relationships of the groups to which these cercariae belong. It is not an example of convergence as upheld by some authors. The presence of stylet in Macrocercariae is a secondary character and should not be considered as showing relationship of the Gorgoderidae with the Allocreadioidea Nicoll. The stylet is absent in Rhopalocercariae and the Macrocercous cercaria, *C. mitocerca* Miller, 1936 which according to Fischthall (195.), represents the ancestor of Rhopalocercariae and which with Macrocercariae and Microcercaria *C. phyllodistomum folium* (Sinitsin 1901), arose from a common unknown ancestor ; the divergence having occurred giving the Rhopalocercariae along one line and the Macrocercariae resulting in the Microcercaria, *C. Phyllodistomum folium* of Sinitsin along the other.

In the above paper, Mehra has created the Superfamily Gorgoderoidea to accommodate the family Gorgoderidae which is characterised by Macrocercous cercariae and Rhopalocercous cercariae. In his opinion the Superfamily Gorgoderoidea though more closely related to the Superfamily Azygioidea Skrjabin and Guschanskaja, 1956 and Superfamily Transversotrematoidea La Rue, 1957 of the Suboder Azygiata La Rue, 1957 and the Superfamily Hemiuroidea Faust, 1929 of the Suborder Hemiurata Skrjabin and Guschanskaja, 1954 included under

the order Hemiuratoidea Mehra, 1957 than to any other digenetic group, differs from them so much as to be created a new order Gorgoderida for its reception.

As regards the evolution of digenea Mehra has reiterated his 1957 view that it has taken place in three divergent lines from the primitive digenetic ancestor : first branch giving the orders Fasciolatoidea Szidat, 1936 ; Plagiorchiida La Rue, 1957 ; and Opisthorchiida La Rue, 1957; the second branch giving the order Strigeatoidea La Rue, 1957 and the third line giving the orders Azygiatoidea, Mehra, 1957 ; Hemiuratoidea Mehra, 1957 and Gorgoderida Mehra, 1958. The order Gorgoderida stands near the base of the third phylogenetic branch.

INTERRELATIONSHIPS AND TAXONOMY OF FURCOCERCOUS CERCARIAE

Interrelationships. — Cercaria is a larval fluke having some potentially adult and some strictly larval characters. The body becomes the adult parasite and possesses an oral sucker and a rudimentary digestive system, usually an acetabulum, a genital rudiment and a simplified excretory system. It may have cystogenous cells or proteolytic glands or both as well as eye spots and other larval structure. The tail is also a larval structure and may be vestigeal, sinple or forked, prehensile or swimming with or without spines and long hairs. It is primarily an organ for passing from the snail host to the next host. In recent years considerable phylogenetic significance has been given to the bifidness or sinpleness of the tail.

Life history studies have shown that members of the families Bucephalidae, Brachylaemidae, Clinostomatidae as well as strigeids and blood flukes all have forked-tailed cercariae, and are closely related to one another. Many studies have also confirmed the view that Spirorchiidae represent the primitive family of blood flukes from where have evolved the degenerate suckerless Aporocotylidae along one line and Schistosomatidae along the other line. Allison (1943) considers that *Leucochloridiomorpha constantiae* represents the living ancestral form of the family Brachylaemidae and that Bucephalata and Brachylaemidae form one group whereas Clinostomata, Strigeata and Schistosomata form another group in the order Strigeatoidea La Rue, 1926. Further, the families Clinostomatidae, Cyathocotylidae represent the primitive forms of Strigeata and Spirorchidae represent the primitive forms of Schistosomata (Mehra, 1950). All these groups of trematodes are closely related to one another through their cercariae and other developmental features. The characters of the larvae and their developmental processes indicate how these groups have radiated from the common ancestor along different lines of adaptations. The common ancestor of the Brachylaemata Mehra, 1950 and Clinostomata Allison, 1943 was an intestinal distome representing the main stem from which the Strigeata La Rue, 1926, Bucephalata La Rue, 1926 and Schistosomata La Rue, 1926 have been evolved along different lines.

Radiative adaptations are the very key note of evolution in the phylogeny of all groups of animals and it equally applies in the case of this group of larval trematodes. Furcocercous cercariae as a group have adapted themselves in a radiating manner with requisite modifications in their morphology and mode of transference from host to host and mode of their further development from a primitive ancestral type. As conceived by Mehra (1950), probably the cercaria of the ancestral fluke from which the Strigeatoidea have evolved along different lines was of the furcocercous type very much like that of *L. constantiae* (Brachylaemidae) with furcae longer or almost as long as the tailstem, and it possessed a pharynx, a

ventral sucker and a simplified excretory system. From this generalized structure the various forms included under the Strigeatoidea have taken different lines culminating in the adults of the families Brachylaemidae, Bucephalidae, Fellodistomatidae Clinostomatidae, Strigeidae and Schistosomatidae (Blood-flukes).

Allison's 1943 researches have shown that Brachylaemid cercaria of *L. constantiae* is of the furcocercous type and in many respects comes nearer to the ancestral type. It has a well developed forked tail with tailstem shorter than the furcae. The cercariae develop in branched sporocysts produced by branched mother sporocysts in the aquatic snail *Campeloma*. Other known cercariae of the family Brachylaemidae are found only in pulmonate snails and have short or vestigeal tails persisting for a short time. The tailless or nearly tailless condition of the cercariae of the other members of the family may be considered as an adaptation to life in terrestrial snails. The cercariae of *L. constantiae* has four pairs of flame cells as in Strigeids and Schistosomes. The tailstem bears long filamentous hairs as in Strigeids and Clinostomes and the metacercariae has a vestigeal reserve excretory system which disappears before the attainment of full size as in the Strigeids and possibly in Clinostomes (Singh, 1959). Baer (1952) states that the development and life history of digenea show that probably they have originated in fresh waters as their developmental stages are invariably found in gastropods and lamellibranchs. Majority of these parasites are still bound by their life cycle to water and consequently the adult worms either partly or wholly to aquatic vertebrates. Adaptations to land vertebrates became possible when metacercariae encysted in aquatic larvae of aerial insects and other organisms and thus became available to hosts *e.g.* bats, birds, etc. Encystment on plants has favoured invasion by trematodes of land vertebrates and appears to be a recent advance. The final passage into the various hosts that are ecologically independent of water became possible with the adaptation of the first larval stages to amphibious or terrestrial snails. In the latter the structure of the cercaria has become modified specially in the tailstem which is usually rudimentary and some times has even disappeared. It is probably on these lines that the loss of tail in other members of the family Brachylaemidae which have terrestrial snail hosts as the first host has taken place and in *L. constantiae* which has aquatic snail host as its first host the tail has been retained. This character appears to be more important both from adaptive and phylogenetic point of view.

The gasterostome cercaria of *Bucephalus polymorphus* is a well known form and has been thoroughly studied, the anatomy by Ziegler (1883), the biology by Wunder (1924) and Wesenberg-Lund (1934), the life cycle and germ cell cycle by Woodhead (1929, 1930 and 1931). The larva may be considered as a specialized furcocercous cercaria in which the tail is much reduced or practically absent and the furca long and strongly developed showing an adaptation to the mode of its pelagic life and its entry into the next host. This modification in the tail allow the cercariae to come rapidly to the surface. The larvae develop in branched sporocysts some of the branches of which appear to be nutritive roots while others are germinal sacs in which germinal masses are seen. In some species rediae are produced while in others germ masses occur in an investing membrane that subsequently break up into germ cells each of which develops into a cercaria. A somewhat precocious redia has been observed as in some Strigeids and schistosomes and this suggests that the so called daughter sporocyst is merely an undeveloped redia.

The bucephalid cercariae resemble the brachylaemid cercariae of *L. constantiae* in the reduced length of the tail stem, their development in branched sporocysts

and in the metacercarial development. The bucephalids resemble the brachylaemids in the smaller size of the ova which are embryonated at the time of deposition, in the miracidia having cilia borne on bars and excretory system. The gasterostome cercariae also resemble the strigeid cercariae in the possession of a pharynx, a group of penetration glands and their development in sporocysts as well as in the metacercarial development. The natatory tail apparatus of bucephalid cercariae also shows a close resemblance to the furcocercous cercariae of the Strigeatoidea in general. The above characters clearly indicate how the two groups of trematodes Gasterostomes and Brachylaemids are interrelated and also point to a close resemblance to the Strigeids in general through their developmental stages.

The position of cercariae belonging to the family Fellodistomatidae has been established by the studies of Cable (1953, 1954c). He traced the life cycle of a Gymnophalline species and concluded that Gymnophalline instead of belonging to the Heterophyidae or Microphallidae should be placed in the Fellodistomatidae. His view is supported by observations on several larval fellodistomatids and particularly the life history of *Fellodistomum fellis* as reported by Chubrik (1952). Cable (1953) thus enlarged the scope of the order Strigeatoidea La Rue, 1926 by assigning the family Fellodistomatidae to that order. He has shown that the Gymnophalline (Fellodistomatidae) have cercariae with forked-tails, simple tails or without tails. The tailstem is with or without paired multiple setae. The excretory system is of the stenostomate type with U shaped or Lyre-shaped bladder having short stem and long broad arms. The penetration glands are numerous and situated in the anterior region. The cercariae develop in simple sporocysts in marine lamellibranchs and the life cycle involves three hosts. The above characters of Gymnophalline cercariae point to a close resemblance with that of the Brachylaemids and Bucephalids, though there exists a significant difference in the adult characters.

La Rue (1957) on the basis of data provided by Cable (1953) includes the family Fellodistomatidae giving it a rank of Superfamily Fellodistomatoidea under the suborder Brachylaimata La Rue, 1957 in the order Strigeatoidea. However, if the adult characters are taken into consideration the Fellodistomatids stand out prominently in contrast to the Superfamilies Brachylaimoidea and Bucephaloidea of the Suborder Brachylaimata. The Fellodistomatids form a well defined and distinct group in the Strigeatoidea having affinity with the Brachylaimidae and Bucephalidae through their larvae. Perhaps, it would be more sound to raise the status of the superfamily Fellodistomatoidea to the rank of a suborder Fellodistomata N. N. under the Strigeatoidea. This would bring the Fellodistomata to an equal footing with the Suborders Strigeata La Rue, 1926 ; Schistosomata La Rue, 1926 ; Bucephalata La Rue, 1926 ; Clinostomata Allison, 1943 and Brachylaemata Mehra, 1950 of the order Strigeatoidea La Rue, 1926.

The cercariae of the schistosomata have developed at least two sets of penetration glands (acidophillic and basophillic) whose secretions enable them to penetrate the tissues of the final host directly. They have become chaemiotacic or haemotropic through adaptation to their respective hosts. The cercariae are bifid with slender tailstem, furca comparatively smaller than the tailstem and constricted off at the base. They are distinguished from all other forked-tailed cercariae by the congenital absence of a pharynx (except in *B. yokogwai*). The apharyngeal condition has probably evolved because of their having become adapted to a mode of life in the blood of the final hosts. The schistosomata may be said to include three groups of cercariae : Spirorchid, Aporocotylid-Sanguinicolid and Schistosome that are closely related to one another.

[132]

The spirorchids are hermaphrodite and live in the blood vascular system of turtles. Some have slender thread like gonads others have enormous testes while one genus has only one. In some cases the acetabulum has disappeared. The intestinal tract resembles that of schistosomes in the absence of a pharynx. The spirorchid cercariae are apharyngeal, brevifurcate, distomate, ocellate with body tailstem and furcae covered with sharp backwardly pointed spines, a head gland in the anterior penetration organ, two kinds of penetration glands witn their ducts capped with papillae or hollow piercing spines. The excretory system is V shaped with flame cells in the tail. The body has a dorsal median crest and the furcae are provided with dorsoventral fin-folds. Both fin-folds and crest are organs of floatation. The carcariae develop in sporocysts.

The lophocercariae of Luhe (1909) and the Lophocerca of Sewell (1922) include cercariae belonging to the families Aporocotylidae and Sanguinicolidae, which develop in the blood vascular system of fishes. Like Spirorchid cercariae they also possess a dorsal median crest, an anterior penetration organ, eye spots (pigmented or unpigmented) and penetration glands of acidophillic or basophillic reactions. The pattern of the excretory system is the same as in tne Spirorchids but there are no flame cells in the tail. The ventral sucker is absent and the cercariae develop usually in simple sac like sporocysts though redial stages have been observed in *S. davisi* by Wales (1958). The monostome cercaria, *C. cristata* of *Sanguinicola inermis* appears to be a specialized form in having a pair of conical spines at the tip of the snout or cone of the bluntly pointed protrusible anterior organ and two caudal excretory canals running through the tail and passing into the furcae to open at the tips. The adults of these families are hermaphrodite with numerous testes as in the Spirorchidae and have a rather unusual H shaped or X shaped gut. They have no suckers and no pharynx and in all these characters resemble to a greater extent the Spirorchids.

The furcocercous cercariae belonging to the Schistosomatidae lack a dorsal median crest though lateral fin-folds on the furcae are present in some species. Roughly they fall in two subgroups : the one that develop in birds and the other that develop in mammals. The cercariae of the avian schistosomes differ from the cercariae of the mammalian schistosomes in having much more elongated bodies and furcae comparatively longer than half the length of the tailstem. However, the presence of pigmented eye spots is a common character of both avian and mammalian schistosomes. The cercariae of the mammalian schistosomes form a well defined group showing great conservatism of excretory system, number and character of penentration glands, nature of the gut, size, and furcae being less than half the length of the tailstem and constricted off from the base. The spines covering the general surface are characteristic as they are usually curved at their tips and penetration glands that are acidophillic and basophillic open through hollow piercing spines. They also possess a head gland in the anterior penetration organ like avian schistosomes and develop in sporocysts. The males in the mammalian species and in some avian schistosomes have gynaecophoric canal in which the females lie for a longer part of their life. In avian schistosomes some species have flattened bodies and others thread like in both the sexes. In recent years most observations made on mammalian schistosomes show all cercariae developing from a single egg are of the same sex, and that while males will reach maturity in the host in the absence of females, the females reach only adolescene in the absence of males. However, when a male enters, the female completes her development even if the male need not be of the same species as the female. Polygamy has also been observed in some species. Further, it has been also

[133]

observed that hybrids can occur although they always resemble the female parent (Cameron, 1958, p. 94).

Wall (1941) considers that Spirorchid cercariae stand intermediate between Lophocercariae (Blood flukes of fishes) and Ocellata Group (Blood flukes of birds and Mammals). He writes, "in this group one would expect cercariae showing all stages of retrogressive development of the dorsal crest leading to those bearing no crest *i.e.* schistosome cercariae." The author is in full agreement with Mehra (1950) that the Spirorchids represent the central generalized stock from where evolution has taken place in two directions towards the Aporocotylidae along one line and along the other towards the schistosomes of birds and mammals in which the furcocercous cercariae have become active swimmers with the loss of the median body crest in general and to some extent furcal fin-folds. The monostome suckerless flukes have evolved from distomes through the reduction and loss of acetabulum or both the suckers respectively. Among the blood flukes many are distomate, some monostomate and some even suckerless and thus are closely related to the strigeids in which certain members have also no acetabulum.

The Clinostome cercariae are furcocercous, lophocercous, brevifurcate, pharyngeal or apharyngeal and have pigmented eye spots and develop in rediae instead of sporocysts. The development of these cercariae in rediae has been considered as a developmental feature having no phylogenetic significance. The cercariae possess long filamentous hairs and spines on the tail and the furcae are without fin-folds. The excretory system is V shaped and the acetabulum represented by a mass of cells somewhat smaller than the anterior penetration organ. They have rhabdocoel gut and penetration glands as in Schistosome and strigeid cercariae. The Clinostome cercariae have several characters in common with schistosome and strigeid cercariae on one hand and with Spirorchid and Lophocercaiiae (Aporocotylid-Sanguinicolid) on the other hand and are also closely related to the Brachylaemid cercariae in general.

The Clinostome cercariae resemble the Lophocercous monostomes in their general appearance, in having dorsal body crest and ill defined acetabulum as well as eye spots. However, they differ in having a pair of flame cells in the tail. They resemble the Spirorchid cercariae in general appearance, excretory system, spination, dorsal crest, outline of the acetabulum and eye spots. These characters bring Clinostome cercariae nearer to the Spirorchid which are supposed to be the primitive schistosomes (Blood-flukes).

Mehra (1950) considers that Clinostomata having brevifurcate, pharyngeal cercariae connect the apharyngeal schistosomata with the pharyngeal strigeata and that they are also closely related to the Brachylaemata. However, the author feels that the nature of pharynx and acetabulum in furcocercous cercariae is of variable nature. Schistosomes lack a pharynx either in cercariae or in the adults but the description of a pharynx by Oiso (1927) in the cercaria of *B. yokogwai* that disappears in the adult is a significant point. There are also certain strigeid cercariae in which the pharynx is obscure but the adults are pharyngeal and in some the pharynx of the cercaria is lost in the adult. A similar condition appears in the case of the acetabulum. As such the nature of pharynx and acetabulum appears to be a common character in the furcocercous cercariae in general.

The Strigeids characterised by the presence of the holdfast organ form a well defined group parasitic in the intestine of vertebrates. The group is considered to be a monophyletic in view of recent investigations and its evolution has been

[134]

fully discussed by Mehra (1950). The group probably originated from the intestinal distome ancestors of the Brachylaimata, Clinostomata, Schistosomata and Bucephalata. The ancestor of this group probably belongs to the family Cyathocotylidae as first suggested by Odhner (1913), later on by La Rue (1926), Szidat (1936) and supported by Mehra (1950). Cyathocotylidae and Clinostomes as stated already are supposed to be primitive types of Strigeata and the life history as studies have proved beyond doubt that Strigeata are closely related to Clinostomata and also to Schistosomata, Bucephalata, and Brachylaemata through their larval stages and developmental features.

Strigeid cercariae present a variety of forms and structure but all are longifurcate having combinations of characters found in other groups of the Strigeatoidea. They are mostly pharyngeal (except few) with pigmented or unpigmented eye spots or none, with variously arranged spines on body and usually long hairs on the tail and some times on the body. The gut has also taken to various shapes and sizes from a small rhabdocoel type to well developed forked intestines reaching behind the ventral sucker or it may be ill defined. The width of the tail is usually equal to that of the body but in some cases it may be bulbous. There may be caudal bodies in the tail or may be absent. The ventral sucker is usually well defined but some times present in the form of a group of cells. The excretory system is V shaped with flame cells in the tail and some times with transverse ducts in the body. Penetration glands and penetration organ are well defined. The cercariae develop in long thread like sporocysts usually with a birth pore. In some strigeids the sporocysts contain a generation of secondary miracidia (Cameron, 1958 ; Premvati, 1955).

In strigeid trematodes parasitic mode of life has entailed modifications in physiology, morphology and life history. The Larvae are free swimming and penetrate directly in the next host to encyst or they may develop without encystment into metacercariae pointing to a close relationship with Clinostomes, Gasterostomes and Brachylaemids. Certain Strigeid cercariae as those of *Apatemon* and *C. letifera* rapidly invade the circulatory system of the next hosts showing a close relationship with the blood flukes. However, the transfer of furcocercous cercariae from the snail to the next host has been achieved in different ways. These cercariae do not possess cystogenous cells as in those of other groups of trematodes. They have only the penetration glands whose secretions enable them to penetrate the tissues of the second host or the final host. In this way they have become chemiotacic or haemotrophic through adaptations to their respective hosts. The cercariae of Brachylaemata, Clinostomata and Strigeata as well as Bucephalata have become adapted after penetration into the second intermediate host to develop into metacercariae with or without encystment. In the Schistosomata on the other hand the cercariae have adapted themselves to penetration in the final host through the skin directly. As such the cystogenous cells in furcocercous cercariae have disappeared and the penetration glands and penetration organs have become strongly developed. Thus the encystment in furcocercous cercariae appears to be a secondary aquisition.

From the above discussions we come to the conclusion that adoption by trematodes of a parasitic way of life has induced changes in all directions and these adaptive modifications have resulted in both convergent and divergent evolution.

Taxonomy : The system of classification proposed for the larval forms (cercariae) by Luhe (1909), Lebour (1912), Cort (1917), Faust (1918, 1924), Sewell (1922), Miller (1926), Dubois (1929) and others in view of recent works on the life history

[135]

and embryology of various groups of digenea have expressed considerable difficulties in the formulation of a taxonomic system for the larval stages. Stunkard (1946) is of the opinion that "these larvae are merely developmental stages in the life cycles of species which belong to diverse families and attempt at classification have limited objectives and little fundamental value. The natural system of classification of digenea must be based on the evolutionary history of the group and further the system must utilize informations afforded by the study of both adults and larval stages and every feature must be evaluated with the greatest care." However, from a study of literature on larval forms belonging to different families of the digenea and from life cycles of many of them, it appears that certain characters such as the number, position and staining reactions of penetration glands, the shape of the bladder, the branching of excretory tubules and flame cell number, the shape and size of body, tail, furcae and their spination, the nature of the stylet, and the character of the germinal mass etc. should be regarded as less important characters for definite specific diagnosis and more important for showing group or subgroup relationships. It is also evident that of the various larval generations, met within the digenea, cercaria is the only stage which shows the greatest resemblance and variety of structures and is most helpful in correlating the adult structures and their phylogeny. The name "cercaria" may be considered as a group name (Dawes, 1956 ; p. 418) and not a generic name and for purposes of descriptions specific names combined with it have nomenclatural validity and may take priority over later names given to the adults of the same species

Taking into considerations the above points an attempt has been made here to present a system of classification of Furcocercous cercariae with special reference to the grouping of Indian forms known so far. In this system, the *Taxa* longifurcate, brevifurcate, monostome, distome, pharyngeal and apharyngeal used by Miller (1926) for grouping of furcocercous cercariae have been dropped and their use for only descriptive purposes has been retained. From life history studies and for reasons already discussed, it has become evident that in purely taxonomic sense these *taxa* have now no great significance. Similarly the various group names created by Sewell (1922) for furcocercous cercariae have been dropped as they have been found untenable in the light of modern researches. For these larvae the only safe and more convenient system of classification appears to assign group names after the name of the families as has been done by previous workers in many cases. Further support in favour of this system is afforded by the life cycles of closely related families and at least one representative from each of the larger groups, whose life cycle is known. As such the following group names of furcocercous cercariae have been suggested :

1. Aporocotylid-Sanguinicolid Group
2. Spirorchid Group
3. Schistosome Group.
4. Clinostome Group.
5. Strigeid Group.
6. Brachylaimid Group.
7. Gasterostome Group.
8. Fellodistomatid Group.

The history and definition of each group and the forms from India known so far representing the above groups shall be communicated in subsequent parts of this series of studies. In this scheme cercariae belonging to the families Azygidae, Beviseculidae and Transversotrematidae which are also in a way furcocercous have not been considered as they differ in several respects from the furcocercous cercariae belonging to the Strigeatoidea La Rue, 1926. They have undergone various modifications from the fundamental pattern of structure characteristic of the

true furcocercous cercariae and are considered to be specialized type of furcocercous cercariae called "Furcocystocercous". These have gigantic body, small furcae, flame cell groups in the tail and develop in rediae.

ACKNOWLEDGMENT

REFERENCES

Allison, L. N. 1943. *Leucochloridiomorpha constantiae* (Muller) (Brachylaemidae), its life cycle and taxonomic relationships among digenetic trematodes. *Trans. Amer. Micros. Soc.* 67 : 127-168.

Baer, J. G., 1952. Ecology of animal parasites. The University of Illinois Press, Urbana.

v. Beneden, P. J., 1858. Memoire zur les vers intestinaux. Etr. Suppl. Comp. rend. Seances acad. Sci. T. 11, 376 pp. Paris.

Bojanus, L. H., 1818. Kurze Nachricht Uber die Cercarien und ihren Fundort. *Isis* (Oken) 1, Col. 729-730 (corrected numbers 688-689).

Bory De St. Vincent, 1889. Histrsonelle, Histrionella. *Dict. Class. Hist. Nat.* 7 : 252-253.

Braun, M., 1889. Vermes Bronn's Kl. *u* Ordnung. Thier Reichs 4, Abt. la, Lief. 9-11, pp. 305-400.

———1892. *Ibid.* Lief 24-27, pp. 737-816.

———1893. *Ibid.* Lief 28-30, pp. 817-925.

Brown, F. J., 1926. Some fresh water larval trematodes with contributions to their life histories. *Parasitol.* 18, 21-34 ; 3 pls.

———1933. On the excretory system and life history of *Lecithodendrium* (Mehl.) and other bat trematodes with a note on the life history of *Dicrocaelium dendriticum* (Rud.) *Parasitol.* 25, 317-328.

Brumpt, E., 1931. *Cercaria ocellata* determinant la dermatite des nageurs, provient d'une bilharzie des canards. *C. R. Acad. Sci., Paris*, 193, 612-614.

Cable, R. M., 1953. The life cycle of *Parvatrema borinquenae* gen. et. sp. nov. (Trematoda:Digenea) and the systematic position of the family Gymnophallinae. *Jour. Parasit.* 39 : 408-421.

———1954a. Studies on the marine digenetic trematodes of peurto Rico. The life cycle in the family Haplosplanchnidae. *Jour. Parasit.* 40, 71-76.

———1954b. Studies on the marine digenetic trematodes of Peurto Rico. The life cycle in the family Magaperidae. *Jour. Parasit.* 40, 202-208.

———1954c. A new marine cercaria from the Woods Hole Region and its bearing on the interpretation of larval types in the Fellodistomatidae (Trematoda:Digenea). *Biol. Bull.* 106, 15-20.

———1956. Marine cercariae of Peurto Rico. Sc. Survey of Peurto Rico and the Virgini Islands. N. Y. Acad. Sci. 16 (pt. 4) 491-576.

Cable, R. M. and Crandall, R. B., 1956. Larval stages and Phylogeny as exemplified by the lung fluke of turtles. Science, 124 (3227), 890.

Cameron, T. W. M., 1958. Parasites and Parasitism, 322 pp. Mathuen and Co. Ltd., London ; John Wiley and Sons, Inc., New York.

Chubrik, G. K., 1952. The larval stage of the trematode *Fellodistomum fellis* Nicoll, 1909 from the invertebrates of the Barentz Sea. *Zoologicheski Zhurnal* 31 (5), 653–558.

Cort, W. W.. 1917. Homologies of the excretory system of forked tailed cercariae. *Jour. Parasit.* 4, 49–57.

———1918. Adaptibility of Schistosome Larvae to new hosts. *Jour. Parasit.* 4, 171-173.

Dawes, B., 1956. The Trematoda with special reference to British and other European forms, XVI-644 pp. Cambridge University Press, Cambridge.

Deising, K. M., 1850. Systema Helminthum, Vol. I, 679 pp. Vindobanae.

———1855. Revision der cercarieen. S. B. Akad. Wiss. Wien, 15 : 377-400.

———1858. Berich.igungen und Zusatze Zur Revision der cercarieen. Sitzber Akad. Wiss. Wien. Math. – *naturw Kl.* 31, 239-290.

Dollfus, R. PH., 1930. Le point d'aboutissement dess Canaux Collecteurs a la vessie chez les distomes ; son importance au point de vue systematique. *Ann. Parasitol. humaine et* Compree 8, 143-146.

———1931. (Clinostomatoidea Comprend Seulement les deux familles des Clinostomatidae et des Nephrocephalidae) Foote Note. *Ann. Parasitol. humaine et* Compree 9, 492-493.

Dubois, G., 1929. Les cercaires de la region Neuchatel. *Bull. Soc. Neuchatel Sci. nat.* 53, 3-177.

Faust, E. C., 1917. Notes on the cercariae of the Bitter Root Valley, Montana. *Jour. Parasitol* 3, 105-123.

———1918a. Life history studies on Montana Trematodes. *Ill. Boil. Monogr.* 4, 7-120.

Faust, E. C., 1919. A Biological Survey of described cercariae in the United States. *Amer. Nat.* 53, 85-92.

———1919c. The Excretory system in Digenea. II. Observation on the excretory system in Distome cercariae. *Biol. Bull.*, 36 : 322-339 ; 10 figs.

———1924. What constitutes a justifiable basis for systematic grouping of larval trematodes ? *Jour. Parasit.* 11, 112-114 ; 2 text figs.

———1929. Human Helminthology. La and Febiger, Philadelphia, 616 pp.

———932. The excretory system as a method of classification of digenetic trematodes. *Quart. Rey. Biol.* 7, 458-468.

De Filippi, F., 1837. Descrizione di nuovi entozoi trovati in alcuni molluschi d' aqua dolce. Bibliotheca Ital., an 22, 87, Sett., pp. 333-340.

———1854. Memoire pour serviro l' histoire genetique des trematodes. 30 30 pp., Turin.

Fuhrmann, O., 1928. Zweite Klasse Cladus Platyhelminthes : Trematoda. In Kukenthal's Handb. d. Zool. 2, 1-14 0.

Lamarck, J. B., 1815. Histoire naturelle des animaux sans vertebres, Vol. 1, XVI + 462 pp., Paris.

La Rue, G. R., 1926. Studies on the trematode family Strigeidae (Holostomidae) No. III. Relationships. *Trans. Amer. Micros. Soc.* 45, 265-281.

[138]

La Rue, G. R., 1938. Life history studies and their relations to problems in taxonomy of digenetic trematodes. *Jour. Parasit.* 24, 1-11.

———1957. Parasitological Reviews. The Classification of Digenetic Trematodes. A review and a new system. *Exp. Parasit.* 6, 306-349.

Lebour, M. V., 1912. A review of the British marine cercariae. *Parasitol.* 4 (4), 416-456.

Leiper, R. T., 1915. Report on the results of the Bilharzia Mission in Egypt. *Jour. Roy. Army Med. Corps* 25, 1-55 ; 147-192 ; 253-267.

Leuckart, R., 1882. Zur Entwicklungsgeschichte des Leberegels (*Distomum hepaticum*). *Arch. Naturgeschieht.* 48, (V. 1), 80-119.

Looss, A., 1896. Recherches sur la faune parasitaire de l' Egypt Premiere-partie *Mem. Inst.* Egypt 3, 1-252.

———1899. Weitere Beitrage zur Kennthis der Trematoden-Fauna Aegyptens, zugleich versuch zu einer naturlichen Gliederung des Genus Distomum Retzius. *Zool. Jahrb.* 12, 521-784.

Luhe, M., 1909. Parasitischer Plattwurmer 1. Trematodes Susswasserfauna Deutschlands (Brauer) Heft 17, iv + 217 pp.

Mehlis, E., 1931. Novae observationes de entozois. Auctore Dr. Fr. Chr. H. Creplin *Isis* (Oken) (1), pp. 68-99, 166-199.

Mehra, H. R., 1950. Evolution of the blood flukes and strigeid Trematodes. Presidential Address. Business Matters, National Acad. Scien., India, 1950.

———1957. The classification of Digenetic Trematodes (Abstract). Proc. Nation. Acad. Sci., India 27th Ann. Session, 1957.

———1958. On a New Trematode *Staphylorchis scoliodonii* n. sp. (Family Gorgoderidae Looss, 1901 : Subfamily Anaporrhutinae Looss, 1901) with phylogenetic discussion and classification of the Gorgoderidae. *Ann. Number, Nation. Acad. Sci., India*, 1958, pp. 87-88.

Miller, H. M., 1923. Notes on some furcocercous larval trematodes *Jour. Parasit.* 10, 35-46.

———1926. Comparative studies on furcocercous cercariae. *Ill. Biol. Monogr.* 10, 265-370 ; 8 pls. , 2 text figs.

———1927. Furcocercous larval trematodes from San Juan Island, Washington. *Parasitol.* 19 (1), 61-82.

Miller, E. L., 1936. Studies on North American Cercariae. *Ill. Biol. Monogr.* 33, 7–122.

Monticelli, F. S., 1888. Saggio una morphologia dei trematodi vii + 3-130 pp., Napoli.

———1892. *Cotylogaster michaelis* n. g., n. sp. e, revisione degli Aspidobothridae. Festschriff Siebenzigs Geburtstag R. Leuckart's pp. 168-214.

Muller, O. F., 1773. Vermium terrestrium et fluviatilium. 1, Vermes, 11 pp.

Nicoll, W., 1934. Vermes. *Zoological Records* (1933), 70, Division, VI, 137 p.

Nitzsch, C. L., 1817. Beitrag zu Infusorienkunde Oder Naturbeschreibung der zerkarion und Bazillarien. N. Schrift. Naturf. Gesellsch, Halle 3, viii + 128 pp.

Odhner, T., 1905. Die trematodes des arktischen Gebietes (In Römer und Schaudinn. Fauna Arctica, 4, 2, Lief, pp. 291-372).

————1912. Zum naturlichen system der digenen Trematoden V. Die phylogeniedes Bilharzia-Types. *Zool. Anz.* 41, 54-71.

————1913. *Ibid.* 42, 289-318.

Oiso, T., 1927. On a new species of avian schistosome developing in the portal vein of the duck and its life history. (Japanese text). Taiwan Igakkwai Zasshi, Taihoku (270) pp. 848-865 (English Abstract, pp. 5-7).

Poche, F., 1926. Das System der Platodaria. *Arch*. Naturgeschichte Abt, A 9 (2-3), 1-458.

Porter, A., 1938. The larval trematodes found in certain South African mollusca with special reference to Schistosomiasis (Bilharziasis). *Publs S. African Inst. Med. Res.* 3 (42), 492 pp.

Premvati, 1955. *Cercaria multiplicata* n. sp. from the snail *Melanoides tuberculatus* (Muller). *Jour. Zool. Soc. India* 7, 13-24.

Railliet, A., 1919. Nouveaux trematodes du chien. (Abstr. of Hall, M. C. and Vidgor, M., 1919). Rec. m'ed Vet, 95, 229-232.

Rothschild, M., 1935. The trematode parasites of *Turritella communis* Lmk. from Plymouth and Naples. *Parsit*. 27, 152-170.

Sewell, R. B. S., 1922. Cercariae indicae. Ind. Jour. Med. Res. 10, 1-372, 32 pls., 7 text figs.

V. Siebold, C. T. E., 1835. Helminthologische Beitrage. Arch. Naturgeschichte 1 J. 1, 45-84.

— — — 1850. Uber den Generationswechsel der Cestoden nebst einer Revision der Gattung *Tetrarhynchus*. *Z. Wiss Zool.* 2, 198-253.

Singh, R. N., 1955. Studies on a new species of schistosome cercaria from *Lymnaea luteola* f. *australis*. Proc. Nation. Acad. Sci., India 25 (Sec. B), 23-28.

————1959 Studies on the morphology and life history of *Clinostomum piscidium* Southwell and Prashad, 1918 (Trematoda : Clinostomatidae). *Proc. Nation. Acad. Sci., India* 29, (Sec. B), 12-33.

Sinitsin, D. T., 1911. Parthenogenetic generation of Trematodes and its progeny in the mollusca of the Black Sea. *Rec. Acad. Sci. St. Petersburg* (8), 30 : 1-127 ; 6 pls. (Russian).

Skrjabin, K. I. and Guschanskaja, L. K. H., 1954. Suborder Hemiurata (Markevitsch, 1951) Skrjabin et Guschanskaja, 1954. (In Skrjabin. K. I., Trematodes of animals and man. *Moskva*, Vol. 9, 227-653.

————1956. Systematics of the Trematodes of the suborder Hemiurata (Markevitsch, 1951) Skjabin et Guschanskaja, 1954. Russian text. Trudy Gelmin Lab. Akad. Nauk. S.S.S. R8, 144-158.

Sonsino, P., 1892. Studi sui parassiti molluschi di aqua dolce dintorni di Cairo in Egitto. Leuckart's Festschr, 134-147.

Soparkar, M. B., 1921. Notes on some furcocercous cercariae from Bombay. *Ind. Jour. Med. Res.* 9, 23-32, 4 pls.

Steenstrup, J., 1842. Über den Generationswechsel, order die Fortpflanzung und Entwicklung durch abwechselnde generationen, eine eigenthumliche from der Brutpflege in der niederen Thierclassen. (Translation by C. R. Lorenzen), 140 pp, Copenhagen.

Stiles, C. H. W. and Hassal, A., 1908. Index-Catalogue of medical and veterinary Zoology. Subjects : Trematoda and trematode diseases. Bull (37) Hyg. Lab. U. S. Pub. Health and Mar. Hospit. Service, 401 pp.

————1926. Key Catalogue of worms reported for man. Bull. (142) Hyg. Lab. U. S. Pub. Health Ser., 66-196 pp.

Stiles C. H. W. and J. Goldberger, J., 1910. A study of the anatomy of *Watsonius watsoni* (n. g.) of man and of Mineteen allied species of mammalian trematode worms of the Superfamily Paramphistomoidea. Bull. (60) Hyg. Lab. U. S. Pub. Health and Marine Hosp. Ser. 259 pp.

Stunkard, H. W., 1923. Studies on North American Blood flukes. *Bull. Amer. Mus. Nat. Hist.* 48, 165-221.

————1929a. The excretory system of *Cryptocotyle* (Heterophyidae). *Jour. Parasit.* 15, 259-266.

————1929b. Parasitism as a biological phenomena. *Sci. Monog. N. Y.,* 28, 349-362.

————1934. The life history of *Typhlocaelum cymbium* (Diesing, 1850) Kossak, 1911 (Trema toda : Cyclocaelidae), a contribution to the phylogeny of monostomes. *Bull. Soc. Zool. Fr. Fr.* 59, 447-466.

————1940. Life history studies and the development of Parasitology. *Jour. Parasit.* 26, 1-15.

————1945. The morphology of *Tamerlania bragai* Dos Santos, 1934. *Jour. Parasit.* 31, 301-305.

————1946. Interrelationships and Taxonomy of the Digenetic Trematodes. *Biological Reviews* 21, 148-158.

Szidat, L., 1932. Uber cysticerke Riesencercarien, insbesondere *Cercaria miriabilis* M. Braun and *Cercaria splendens* n. sp. und ihre Entwicklung im Magen von Raubfischen zu Trematoden der Gattung *Azygi* a Looss. *Z. Parasitenk.* 4, 447-505, 22 figs.

————1936. Uber die Entwicklungsgeschichte und den erten Zwischenwirt von *Paramphistomum cervi* Zeder, 1790 aus dem Magen von Wiederkauern. Zeitschr. *Parasit.* 9 (1), 1-19.

————1936a. Parasiten aus Seeschwalben I. Uber neue Cyathocotyliden aus dem Dram von *Sterna hirundo* L. und *Sterna paradisea. Z. Parasitenk.* 8, 285-316.

Szidat, L., 1939. Beitrage zun Aufbau eines naturlichen systems der Trematoden. I. Die Entwicklung von *Echinocercaria choanophila* U. Szidat zu *Cathaemasia hians* und die Ableitung der Fasciolidae von den Echinostomidae. *Z. Parasitenk* 11, 239-283.

Talbott, S. B., 1936. Studies on schistosome dermatitis II. Morphological and life history studies on three dermatiting producing schistosome cercaria C. *elva* Miller, 1923 ; C. *stagnicolae* n. sp. and C. *physellae* n. sp. *Amer. Jour. Hyg.* 23, 372-384.

[141]

Thomas, A. P. W., 1883. The life history of liver fluke (*Fasciola hepatica*). *Quart. Jour. Micros. Sci.* n. s. (89), 23 : 99-133.

Timon-David, J., 1955. Cycle evolutif dum trematode cyclocoelidae, *Pseudhyptiasmus dollfusi* Timon-David, 1950 Recherches experimentales. *Ann. Parasit. hum. et Comp.* 30, 42-61.

Talor, E. L. and Baylis, H. A., 1930. Observations and experiments on a dermatiting producing cercaria and on other cercariae from *Lymnaea stagnalis* in Great Britain. *Trans. Roy. Soc. Trop . Med. Hyg.* 24, 219-244.

Wall, L. D., 1939. Life history of *Spirorchis* sp. (Trematoda : Spirorchiidae). *Jour. Parasit.* 56 (6), Suppl. 28.

———1941a. Life history of *Spirorchis elephantis* (Cort, 19 7) a new blood fluke from *Chry emis picta*. *Amer. Mid. Natur.* 25, 402-412.

———1941b. *Spirorchis parvus* (Stunkard, 1923), its life history and the development of its excretory system. *Trans. Amer. Micros. Soc.* 60, 221-260.

———1951. The life history of *Vasotrema robustum* (Stunkard 1928), Trematoda : Spirorchiidae. *Trans. Amer. Micros. Soc.* 70, 173-184.

Wallace, H. E., 1941. Life history and embryology of *Triganodistomum mutabile* (Cort) (Lissorchiidae : Trematoda) *Trans. Amer. Micros. S c.* 60, 309-326.

Wesenber-Lund, C. J., 1934. Contributions to the development of the Trematoda Digenea. Part II. The biology of the fresh water cercariae in Danish fresh waters. *Kgl. Danske Videnskab. Selskab, Skrifter Naturvitenskab Math. Afdel.* [9 Raekke.] 5 (3), 1-223.

Woodhead, A. E , 1929. Life history studies on the trematode family Bucephalidae. *Trans. Amer. Micros. Soc.* 48, 256-275.

———1930. *Ibid*. II. *Trans. Amer. Micros. Soc.* 49, 1-17.

———1931. The germ cell cycle in the trematode family Bucephalidae. *Trans. Amer. Micros. Soc.* 50, 169-188.

———1936. A study of the gasterostomes cercariae of the Huron River. *Trans. Amer. Micros. Soc.* 55, 465-476.

Wright, C. A., 1956. Studies on the life history and ecology of the trematode genus *Renicola* Cohn, 1904. *Proc. Zool. Soc., London* 126, 1-49.

Wunder, W., 1924. Die Schwimmbewegung von *Bucephalus polymorphus* v. Bear. *Z. vergl. Physiol.* 1, 289.

Ziegler, H. E., 1883. *Bucephalus* and *Gasterostomum*. *Z. Wiss. Zool.* 39, 537-571 ; also *Zool. Anz.* 6th year pp. 476.492.

1, 2 and 3—These reference were not consulted in original.

1 Consulted from Lal, M. B., 1957. A new approach to Helminthology in India. Presidential Address. Proc. Indian Science Congress, Section of Zoology and Entomology, pp. 108-122.

2 and 3 Consulted from Mehra (1958).

ON A NEW TREMATODE *STAPHYLORCHIS SCOLIODONII* n. sp.
(FAMILY GORGODERIDAE LOOSS, 1901 ; SUBFAMILY
ANAPORRHUTINAE LOOSS, 1901) FROM SOUTH
INDIAN SHARK *SCOLIODON SORRAKOWAH*
WITH A PHYLOGENETIC DISCUSSION
AND CLASSIFICATION OF THE
FAMILY

By

H. R. MEHRA

Allahabad

The little known genus *Staphylorchis* Travossos, 1920 prasitic in the body cavity of the Selachii of Indian and Australian seas created for *Petalcdistomum cymatode* Johnston, 1913 separating it from *Petalodistomum polycladum* Johnston, 1913, the two species for which he created the genus *Petalodistomum* parasitic in the body cavity of the sting-ray *Dasybatus kuhlii* of the Australian seas has been recently studied closely. Travossos thought that his new genus which resembles more *Anaporrhutum* Looss, 1901 than *Petalodistomum* is characterised by its highly sinuous intestinal caeca and the disposition of its testes.

Lühe in 1906 described a new species *Anaporrhutum largum* for a single specimen obtained from the body cavity of *Rhinoptera javanica* Muller and Henle at Kalpitiya, Ceylon. Travossos in 1922 in his revision of the family Gorgoderidae included it in the genus *Anaporrhutum*, but Baylis in 1927 assigned it to the genus *Staphylorchis* as *Staphylorchis largus* on the basis that the testes in *Anaporrhutum* overreach the intestinal caeca and extend beyond them towards the median line, whereas in Luhe's species they are entirely extra-caecal. Southwell (1913) recorded *Staphylorchis largum* (Lühe, 1906) Baylis 1927 from the coelom of Selachian fishes of Ceylon, *Chiloscyllium indicum, Gingly-mostoma concolor* and *Aetobatis narinari* and also compared with it a species of *Anaporrhutum* obtained by Dr Jenkins from the coelom of *Stegostoma tigrinum* caught off the Orissa coast in 1910. Chaudhuri (1916) collected *A. larguum* also from the coelom of *Hypolophus sephen* from the Chilka lake. Nagaty (1930) Studied about 40 worms contained in a bottle labelled "Parasites from *Ginglymostoma concolor* Periya Paar, Pearl Banks, Ceylon", which he identified as *Staphylorchis largum* (Luhe, 1906) Baylis, 1927. He was able to supplement Lühe's description of this species from the large number of specimens available. He was in agreement with Baylis in the allocation of this species to the genus *Staphylorchis*. Dollfus (1937), however, criticis-ed the position of *largum* in the genus *Staphylorchis*. He expressed the view that it stands intermediate between *Anaporrhutum, Staphylorchis* and even *Petalodistomum*, and thought that it may be justifiable to consider it as subg enus. It may be mentioned that Luhe (1906, p. 105) had expressed the opinion that while *Probolitrema* may be accepted as a separate genus, it would not be proper to create a third genus for *largum* on account of its entirely intracaecal vitellaria ard entirely extracaecal testes.

Dollfus's remark that in *Staphylorchis* the bifurcation of the excretory bladder is near the termination of the caeca is based on the apparently erroneous observation of Johnston in *S. cymatodes*. We may mention that in *Staphylorchis* as in *Anaporrhutum*, *Probolitrema*, *Petalodistomum*, *Nagmia i.e.*, all the genera of the subfamily Anaporrhutinae Looss, 1901 the exretory vesicle is tubular and bifurcates much in front of the terminal ends of caeca near or a little distance behind ovary It appears that Johnston's figure of *S. cymatodes* does not show correctly the position of the bifurcation of the excretory vesicle. Samuel (1952) in her account of *Staphylorchis gigas* has correctly given this position, which is corroborated by Crusz (1957) in *S. parisi* and our own observation in the case of the new species descibed here. The testes are, however, extracaecal in *largum*, though they occupy a small area and do not extend as in the other species of *Staphylorchis* between the level of acetabulum and that of the termination of the caeca.

Samuel (1952) has given a fairly detailed account of her new species *S. gigas* parasitic in the coelom of shark *Galeocerdo tigrinus* collected near Madras and Nagapattinam, Bay of Bengal. She while discussing the systematic position of Lühe's species *largum* follows *Dollfus* and comes to the conclusion that it should be included in the genus *Anaporrhutum*. We do not agree with this as will be seen later in the systematic discussion of the new species described in this paper. Crusz (1957) has described another new species from the body cavity of the shark *Scoliodon welbeehmi* of Ceylonese waters (Indian Ocean), which as the author mentions is more closely related to *S. gigas* Samuel than to *S. cymatodes* (Johnston). In this paper is described the fifth species of the genus, *Staphylorchis scolidonii* n. sp. obtained from the body cavity of *Scoliodon sorrakowah* of the south Indian seas. Only one specimen was obtained in the course of dissection in the B. Sc. class room from one shark.

Length of formalized specimen 9 mm. ; maximum breath at about middle of the body length 8 mm. It resembles *S. cymatodes* in size and differs remarkably from *S. gigas* Samuel and *S. parisi* Crusz, which are very large. Body moderately large, flattened, almost spherical petaloid with posterior part broad and rounded, fairly fleshy, smooth without spines or scales ; anterior end narrower and slightly projecting, 0·255 mm. in breadth, posterior end 0·42 mm. in breadth. Oral sucker nearly terminal, with the opening slightly ventrad at anterior end, larger and stouter than ventral sucker, broader than long, measuring 1·4025 mm. in maximum breadth by 0·8075 mm. in antero-posterior length; breadth and length of its opening 0·2975 mm. and 0·0875 mm. respectiyely. Ventral sucker also much broader than 1·2165 mm. in maximum breadh and 0·82 mm. in length, situated a little behind long, oral sucker at about one sixth body length distance from anterior end and 0·153 mm. behind intestinal bifurcation. The openings of the suckers are transverse ; the distance between the two suckers is 0·765 mm.

Pharynx strongly muscular, situated immediately behind oral sucker projecting anteriorly a little under its posterior wall, measurings 0·5185 mm. in breadth by 0·4335 mm. in length. Oesophagus absent ; intestinal caeca simple without diverticula, diverge at their origin just behind pharynx making almost a semicircular arc at the wide intestinal bifurcation ; intestinal caeca wide, situated midway between the middle longitudinal axis of the worm and its lateral borders are strongly winding being thrown into a number of undulations, terminating a little in front of hinder end with terminal ends facing each other ; number of undulations in both caeca almost equal, 7 in one and 8 in the other.

Genital opening situated median, 0·51 mm. distance in front of intestinal bifurcation ventrally to posterior margin of pharynx. In the type species *S. cymatodes*

(Johnston) it lies in the middle line between intestinal fork and anterior edge of ventral sucker. In *S. gigas* Samuel, 1952 and *S. parisi* Crusz, 1957 it also lies median on the ventral surface a short distance behind intestinal bifurcation. Its position in the new species in front of the intestinal fork behind posterior margin of pharynx, however, resembles that in *S. largum* Lühe, (1906).

Excretory opening terminal at hinder end, surrounded by a sphincter, leads into a tubular excretory bladder which extends forwards between intestinal caeca and bifurcates at about the middle of body length at a little distance behind ovary and receptaculum seminis into two long branches one on each side running forwards a somewhat tortuous course and each bifurcating again just outside or underneath its respective intestinal caecum into a forwardly and backwardly running branches.

Testes divided into a large number of small follicles of almost oval or rarely spherical shape irregularly distributed and occupying on each side a fairly broad area outside intestinal caeca between the latter and lateral edges of the body extending from about 1½—2 mm. distance behind ventral sucker to a little in front of hinder ends of caeca. Testes follicles measure 0·068-0·11 by 0·051-0·068 mm. in size. Their number could not be counted as they are smaller and more numerous than in other species. In *S. cymatodes* in which they are about 50 small rounded follicles on each side, they measure 0·107-0·129 mm. in diameter. In *S. gigas* and *S. parisi* they are 40-52 in number on either side in the former and 45-61 in the latter, measuring 0·8 by 0·6 or 0·4-0·8mm. in the former and 0·06-1·5mm. by 0·5-1·0 mm. in the latter. In these three species they extend anteriorly to the level of acetabulum in contrast to that in the new species in which they commence about one fifth body length distance behind it. In *S. largum* (Luhe, 1906) their number is very small, 14 on the right and 17 on the left according to Lühe and 9-21 on each side according to Nagaty. In this species they occupy only a small area much behind acetabulum and in front of terminal ends of straight or slightly sinuous caeca. According to Baylis (1927) their diameter is 0·2-0·34 mm. The vasa efferentia were not clearly seen ; they were seen to arise from the testes in a few places joining up in groups of more than 5 or 6 to enter the long vas deferent duct. The two vasa deferentia join just behind anterior wall of acetabulum dorsally to the right side of the latter to form the tubular vesicula seminalis which extends in front to the right side towards intestinal bifurcation. The terminal end of the vesicula seminalis is not swollen into an elongated oval sac as in *S. gigas* ; nor is it dilated into a cirrus like end organ as in *S. parisi*. It passes more or less insensibly into slightly narrower terminal ejaculatory duct which lies to the right side opposite the terminal part of metraterm.

Ovary small, much lobed, mulberry-shaped or rather follicular composed of small closely set follicles, 0·153-0·233mm. in size, situated to left side of median line in close contact with left side of receptaculum seminis with vitelline reservoir ventral in its anterior zone. Receptaculum seminis, rounded, 0·7mm. in diameter, situated almost median directly behind acetabulum, at about 0·05 mm. distance behind it and in close contact with right vitelline gland, at 0·072 mm. distance from the loop of right intestinal caecum and 0·782 mm. distance from the loop of left caecum. It is much smaller than that of *S. gigas* and *S. parisi* ; in the latter species it is very large, almost double the size of either of the suckers. The two vitellaria, receptaculum seminis and ovary with the anteriorly lying shell gland mass and vitelline reservoir and the transverse vitelline ducts are intercaecal as in other species of the genus lying a little distance behind acetabulum, closely behind

first prominent loop of intestinal caeca. Each vitelline gland is composed of a loosely and irregularly arranged winding cord of vitelline cells in the form of a network of a few branched tubes and not of follicles. The right vitelline gland, 0·765 mm. in breadth and 0·34 mm. in length lies a little more anteriorly, occupying almost the entire space between right intestinal caecum and receptaculum seminis to the right side of anterior half of the latter ; left vitelline gland lies between ovary and left intestinal caecum opposite and slightly at a lower level to the right gland, measuring 0·85mm. in breadth and 0·51 mm. in length. The vitellaria resemble in shape those of *S. cymatodes* in which each gland consists of "a much branched tube rather than of follicles" differing from the condition in *S. gigas* in which "each gland consists of a cluster of branching follicles which resemble clumsy finger like projections with rounded extremities". In *S. parisi* they are dendritic and each is about half the size of the enormously large receptaculum seminis. Transverse vitelline ducts conspicuous, the right one placed a little in front of the left one, the former situated ventral to anterior part of receptaculum seminis. Vitelline reservoir at anterior margin of ovary just behind shell gland mrass near left side of receptaculum seminis ; shell gland mass contiguous and anteiior to ovary. Uterus arises from between receptaculum seminis and ovary, passes coled towards left intestinal caecum dorsally just behind left vitelline gland to take its descending course, reaching posteriorly to about half the distance between acetabulum and hinder end of body to a little behind the middle of body length in the intercaecal area, coils not crowded but on the other hand loosely arranged, ascending coils passing to left side to enter terminally the long metraterm situated between left vitelline gland and ovary. Metraterm long forming a somewhat coiled mass near and infront of ovary behind acetabulum between receptaculum seminis and left intestinal caecum, taking terminally a forward course overlapping acestabulum to left side and running median in front of the latter opposite to terminal part of vesicula seminalis and ductus ejaculatorius to open at the genital pore. Ova numerous, oval, yellow brown and 0·0374-0·0467 by 0·0187-0·0-8 mm. in size. The uterus differs from that of other species in extending posteriorly to a little behind the middle and not to the hinder end or to blind ends of caeca and also in the configuration of its coils. It is not voluminous as in *S. gigas* and *S. parisi*. The metraterm in the new species is much longer and the ova smaller than in other species.

SYSTEMATIC DISCUSSION

The genus *Staphylorchis* created by Travassos in 1920 to accomodate *Petalodistomum cymatodes* (Johnston, 1913) and based on the strongly winding character of intestinal caeca, entirely intracaecal position of vitellaria and follicular condition and entirely extracaecal position of testes should include *Anaporrhutum largum* (Lühe, 1906) as assigned by Baylis in 1927. Though the caeca in this species are almost straight or slightly winding, the position of its vitellaria and testes follicles entitle it to that position. It cannot be included in the genus *Nagmia* Nagaty, 1930 as sugested by Yamaguti (1953) on account of its simple caeca without outgrowths, prefurcal position of the genital opening and the testes not forming grape like symmetrical bunches of acini as in *Nagmia yorkei*. The testes in the latter species are also larger in number 29-35 in contrast to 9-21 of *S. largum*. In *Nagmia* the excretory bladder has lateral branches along its length and the receptaculum seminis is very large. The relative position of the latter to the ovary and acetabulum in *S. largum* is, however, similar to that in *Nagmia*, which also resembles it in the acetabulum being larger than the oral sucker. Lühe (1906) was reluctant
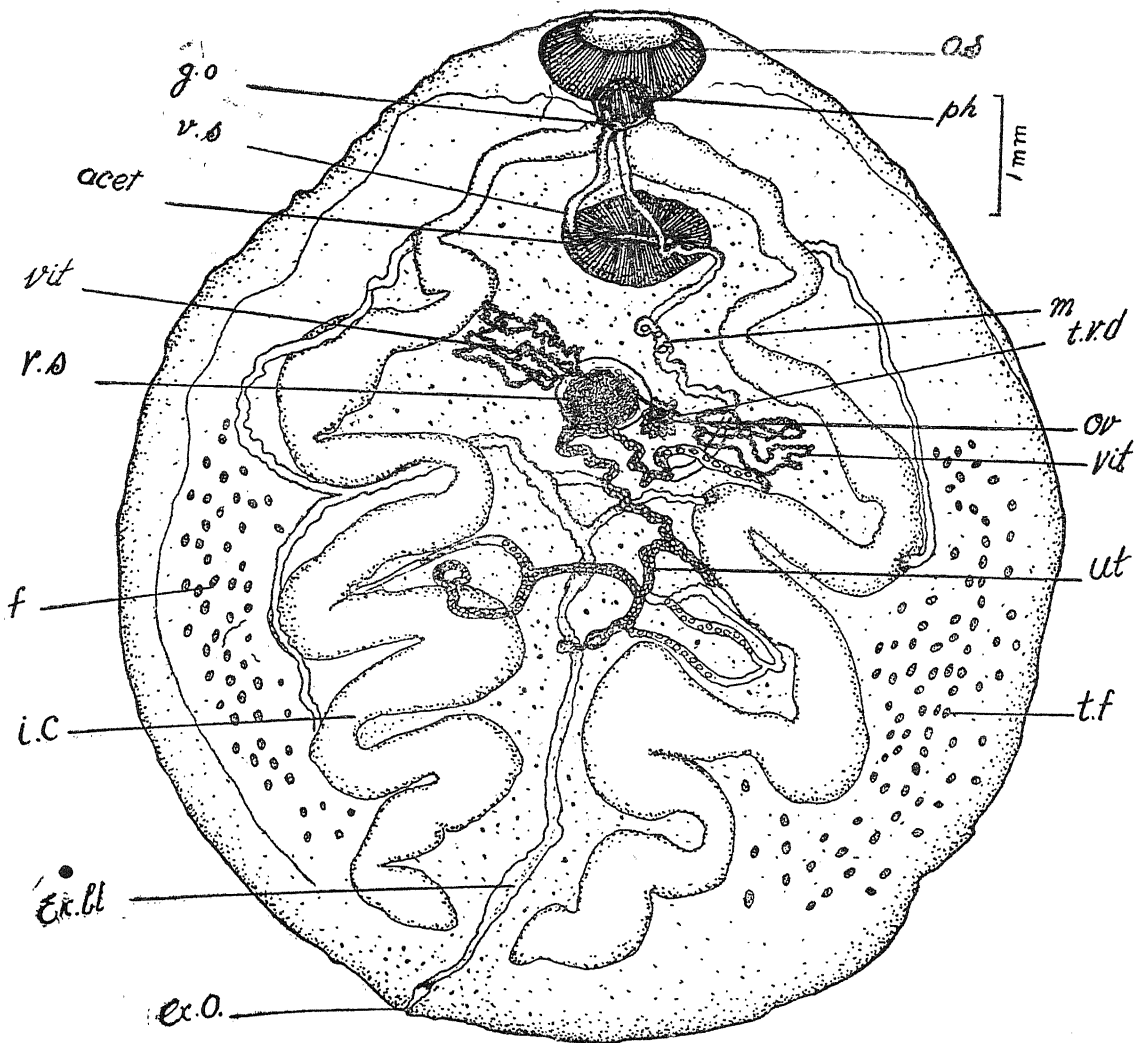
Fig. 1—*Staphylorchis soliodoni* n. sp. ventral view, acet. actabulum ; ex. bl. excretory bladder ; ex. o. excretory opening g. o. ; genital opening ; i. c. intestinal caecum ; m. metraterm ; o. s. oral sucker ; ov. ovary ; ph. pharynx ; r. s. receptaculum seminis ; t. f. testis follicles ; t.v.d. transverse vitalline duct ; ut. uterus ; vit. vitelline gland ; v. s. vesicula seminalis.

to create a third *genus* for his species *largum* and therefore included it in *Anaporrhutum* Ofenheim, 1900 as held separate from *Probolitrma* Looss, 1902. He, however, thought it preferable to consider the latter genus as a subgenus rather than a separate genus. Dollfus (1937) criticising Baylis's assignment of *largum* to *Staphylorchis* expressed the view that it stands intermediate between *Anaporrhutum, Staphylorchis* and *Petalodistomum* and thought it possible to create a subgenus for it. He also expressed that *Nagmia* Nagaty may be considered as a subgenus of *Petalodistomum* rather than as a separate genus, the only difference being that in *Nagmia* the testes are separated into a large number of separate follicles. Samuel (1952) follows Tra-

[147]

vassos (1922) and Dollfus (1937) in recognising the position of *largum* in *Anaporrhutum*. We may point out as previously done by Baylis that the genus *Anaporrhutum* is clearly distinguished by the position of its testes which consist of symmetrical grape like bunches of numerous small follicles overreaching caeca and extending inwards to them towards median line. The testes in this genus are partly extracaecal and partly intracaecal and the vitellaria lie just inside and ventral to the caeca, whereas in *S. largum* testes are entirely extracaecal and vitellaria entirely intercaecal. In these features as well as in the prefurcal position of the genital opening it stands quite apart from *Anaporrhutum*, though the almost straight or slightly sinuous caeca bring it close to the latter genus. *S. scoliodonii* n. sp. connects *S. largum* with other species of *Staphylorchis* in which genital pore occupies a postfurcal position. In veiw of all this the position of *S. largum* in the genus *Staphylorchis* must be considered as valid. There may be some force in the points of view of Luhe (1906) and Dollfus (1937) that *Anaporrhutum, Probolitrema. Petalodistomum, Staphylorchis* and *Nagmia* may be considered as subgenera, but we consider it proper to maintain them as separate genera on account of their well marked differences.

S. scoliodonii n. sp. resembles *S. largum* (Lühe) in the absence of oesophagus besides the prefurcal position of its genital opening, but it differs in the relative sizes of the suckers. The acetabulum is much larger in *S. largum* about twice the size of the oral suckar according to Baylis. The oral and ventral suckers are equal in *cymatodes gigas*, and *parisi*, but in *scoliodonii* n. sp. oral sucker is a little larger than ventral sucker. *S. largum* differs from the latter and other species of the genus in the receptaculum seminis being situated mostly anterior to the ovary in contact with posterior margin of acetabulum and not laterally to the ovary a little distance behind acetabulum.

S. scoliodonii n. sp, resembles *S. cymatodes* closely in the size of its body, shape, size and position of the ovary, receptaculum seminis and vitellaria, but the testes are smaller in size and larger in number. The testes also differ in commencing some distance about 2 mm. *i.e.*, about one fifth body length behind acetabulum. *S. gigas* differs from the new specis in the much greater size of the body, which is more than twice its size, in the large size of its receptaculum seminis, equal size of suckers, and structure of the vitellaria. *S. parisi* which resembles *S. gigas* in its large size has much larger receptaculum seminis which is very large about double the size of either sucker. The new species differs from all the species in the uterus terminating posteriorly a little behind middle of the body length *i.e.*, much in front of hinder end, in the greater length of its metraterm and smaller size of ova. The intestinal caeca also diverge widely at the bifurcation in an arc-shaped manner unlike that in other species.

Host : *Scoliodon sorrakowah.*

Site : Body cavity.

Locality : Indian Ocean, South Indian waters.

Key to the species of the genus Staphylorchis Travassos

Intestinal caeca slightly slightly sinuous or straight; Acetabulum strikingly large, about twice the size of oral ...... *S. largum* (Luhe) Baylis, 1927.

Intestinal caeca strongly sinuous ; suckers equal or oral sucker slightly larger
.. (1)

1. Genital pore prefurcal ; uterus not reaching hinder end ; oral sucker larger
...... *S. scoliodonii* n. sp.

Genital pore postfurcal ; uterus reaching hinder end ; suckers equal.
.. (2)

2. Size small, 10·5 mm. long, 8mm.. broad ... *S. cymatodes* (Johnston) Travossos, 1920.
   Size large, 25-40 mm. long, 20-30 mm. broad   .. (3)

3. Ovary slightly lobed ; receptaculum seminis 2·8 mm. in diameter . . . . .
   S. gigas Samuel, 1952.
   Ovary mulberry shaped, receptaculum seminis 5-6 mm. by 3-5 mm. about double the size of either sucker . . . . . . *S. parisi* Crusz, 1957.

It is difficult to ditermine the diagnosis of *Staphylorchis* sp. Dub. from the meager description available as given by Crusz (1957). It appears to be a new species, resembling *S. gigas* on the one hand and *S. cymatodes* on the other.

PHYLOGENETIC DISCUSSION AND CLASSIFICATION OF THE FAMILY
GORGODERIDAE LOOSS, 1901

The position of the Gorgoderidae in the taxonomic system of the Digenea has not been determined as yet. Nicoll (1934) in the Zoological Record VI. Vermes placed it under Dicrocoelioidea. La Rue (1957) assigned it provisionally to the Allocreadioidea Nicoll, 1934. He remarked at the same time "The Allocreadioidea as set up in this system contains such a variety of cercarial types as to present strong indication that the superfamily is not a natural one, and that it should be subdivided into several superfamilies, all more or loss closeley related." On a close examination of the cercarial types—Macrocercariae and Rhopalocercariae which develop in Lamellibranchs of the families Sphaeriidae, Dreissenidae and Unionidae (Rhopalocercariae in Unionidae and Macrocercariae in Sphaeriidae and Dreissenidae) and life cycles and the adult morphology characteristics of the family we consider it necessary to create a new superfamily Gorgoderoidea under a new order Gorgoderida to accomodate the Gorgoderidae. The Cystocercous Macrocercariae and Rhopalocercariae possess long cylindrical unforked tail with its anterior end or basal part hollowed out forming a cavity to accomodate completely or partially the retracted body of the young distome. The tail in the Macrocercaria is considerably longer than the body and its anterior end with the cavity about one tenth or less of the total length. The oral sucker is armed with complicated stylet imbeded in the dorsal wall at right angles to the long axis of the body. In the Rhopalocercariae the stylet is absent and the tail is a little more than half the length of the body. The excretory bladder in both the types of these cercariae as well as in their adults is long, narrow cylindrical or tubular extending forwards to or a little behind posterior margin of acetabulum. Genital opening lies in front of acetabulum ; cirrus sac is absent. Development takes place in sporocysts in clams of the families Sphaeriidae, Dreissen:dae and Unionidae.

Lühe (1909) included in the key of his group of Cystocercous cercariae, *C. mirabilis* M. Braun, 1891, *C. macrocerca* Filippi, 1854 (*Gorgoderinae vitellilobae* Sinitsin, 1905), *C. Gorgoderae cygnoidis* Kowalewski, 1904, *C. Gorgoderae pagenstecheri* Sinitsin, 1905, equivalent and synonymous to *C. macrocerca* of Thiry (1860) as considered by Sinitsin (1905) and Luhe (1909), *C. varscviensis* Sinitsin, 1905 and *C. cystophora* Wagener, 1866. It is now well known that *C. mirabilis* Braun along with *C. wrighti* Ward, 1916, *C. anchorides*, Ward, 1916, *C. macrostoma* Faust, 1918, *C. brookoveri* Faust, 1918, *C. fuska* Pratt, 1919, *C. splendens* Szidat, 1932, are the Furcocystocercous cercariae which develop in rediae with rudimentary pharynx, have two-host life cycle and as shown by Szidat (1932) in *C. mirabilis* and *C. splendens* belong to the genus *Azygia*

[149]

(Family Azygiidae Odhner, 1911, Superfamily Azygioidea Skrj. and Guschanskaja, 1956). *C. cystophora* Wagener as listed by Miller (1936) with *C. appendiculata* Pelseneer 1906, *C. vaullegeardii* Pelseneer, 1906, and several other cercariae for which Sewell (1922) created a separate group 'Appendiculata' and more recent and well described form *C. sinitzini* Rothschild, 1938 belong to the well known group of Cystophorous cercariae which develop in rediae, and have a second intermediate Copepod host in their life cycle. The Cystophorous cercariae belong to the Superfamily Hemiuroidea Faust, 1929 (Families Hemiuridae Lühe, 1901 and Halipegidae, Poche, 1926). Excluding the Furcocercous and Cystophorous cercariae from the Cystocercous group of Lühe the remaining part consists of Gorgoderine cercariae which as separated by Lühe on account of their simple large unforked tails was called by him Macrocrocariae or Macrocercous cercariae, the first known and described cercaria of which is *Cercaria macrocerca* Filippi, 1854. Lühe mentioned that they possess piercing stylet and develop in sporocysts in *Sphaerium*. The latter author however, separated Rhopalocercous Cercariae as a group from Cystocercous Cercariae possibly because he was unaware of the cyst forming character of their tail, though he clearly distinguished them as possessing unforked spineless tails of considerable thickness which when contracted reaches almost or even exceeds breadth of body. Lühe thus by his grouping of the Furcocystocercous, Macrocercous and Cystophorous cercariae in one large group attached importance to the cyst forming character of the tail containing chamber in its basal part in which the larval body can be withdrawn as showing close natural affinity. We are in agreement with him in his estimate of the cystocercous character of the tail of these larvae being of such a unifying and deep seated nature in these three groups so as to justify their inclusion in one group. We do not conceive of this character as an example of convergence.

Cercaria duplicata (Baer, 1827) included by Lühe (1909) in his group Rhopalo-cerke Cercariae and considered by him to develop into *Phyllodistomum folium* is closely related to the Macrocercariae. Fischthal (1951) who made a detailed study of Rhopalocercariae described five new species, all of which develop in clams of the family Unionidae and lack a stylet. *C. mitocerca* described by Miller in 1936 and placed by him with the Gorgoderine cercariae, but differing from them in lacking a stylet, according to Fischthal is a transition form between the Macrocercariae and Rhopalocercariae. It resembles in body features the latter very closely, while its tail has definite features of the Macrocercariae. It also develops like the Rhopalo-cercariae in clams of the Unionidae, whereas the Macrocercariae develop in the so called "finger-nail" clams of the family Sphaeriidae. After comparing certain features of the stumpy tailed microcercous cercaria of *Phyllodistomum folium* described by Sinitzin (1901), Macrocercariae and Rhopalocercariae Fischthal establishes their close relationship and traces their evolution which he represents in a phylogenetic tree. The Macrocercariae, Rhopaocercariae and Microcercaria have arisen from a common unknown ancestor from which the Rhopalocercariae have diverged along one line and the Macrocercariae along the other. A further divergence occurred in the latter group resulting in the Microcercaria *C. Phyllodistomum folium* of Sinitzen (1901) and the large group of Macrocercariae. He considers *C. Gorgoderina attenuata* of Rankin (1939) and *C. Gorgodera amplicava* of Krull (1935) to be the most primitive Macrocercariae in which the entire length of the tail posterior to the anterior chamber is of the same width as the latter.

Sewell (1922) recognized the close affinity of the Macrocercous (Gorgoderine) and Cystophorous groups of cercariae, but he separated the Furcocystocercous cercariae from the Cystocercous Cercariae and included them in Lühe's group of Furcocercous Cercariae under his new "Mirabilis" subgroup named after the first

[150   ]

form discovered, *Cercaria mirabilis* Braun which he considered to form a natural group on account of close similarity of structure and difference in general appearance from other forked-tail forms. He thought that though Luhe included it in the Cystocercous cercariae, it seemed to him that "the cystocercous character of the tail may be merely an example of convergence and not one of true relationship." He mentions that the excretory system of the cercariae of the Mirabilis group has the same fundamental plan as is found in the Furocercous cercariae. Sewell divided the group of Macrocercous cercariae after the separation off from it *Cercaria mirabilis* Braun into three subgroups, "Gorgodera" group, the "Gorgoderina" group, and the "Appendiculata" group. *C. cystophora* according to him exhibits affinities to the "Gorgoderina" group and the "Appendiculata" group. In the latter group he included *C. appendiculata* Pelseneer, 1906, *C. vaullegeardi* Pelseneer, 1906, *C. Indicae* XXXV, Sewell, 1922 and *C. cystophora* Wagener. The excretory system of the latter cercaria he mentions "strongly recalls the arrangement of the excretory canals in such a form *es Gorgodera cygnoides* (vide Looss, 1894. Fig. 125)." His definition of the group Appendiculata can apply to all the Cystophorous cercariae except that *C. vaullegaurdii* Pelseneer and *C. capsularis* as mentioned by Miller differ from the other members of the Appediculata group in that they develop in sporocysts instead of in rediae. Miller (1936) has listed 15 cystophorous cercariae.

Szidat (1932) fully dealt with *C. mirabilis* Braun and *C. splendens* Szidat, 1932 and traced their development in the stomach of predatory fishes into the Trematodes of the genus *Azygia* Looss, 1899. He gave the name *Cystocercaria* to this group of Cystocercous gigantic cercariae in which he also included *C. macrostma* Faust, 1918, *C. brookoveri* Faust, 1918, *C. wrightii* Ward, 1917, and *C. fusca* Pratt, 1919. Sewell had placed these six cercariae in his Mirabilis group. *C. mirabilis* and *C. macrostoma* are the largest among them being 6-7 mm. long, giants among cercariae. Szidat finally decided to enlarge the range of the Strigeatoidea La Rue, 1926 by including the Furcocystocercous cercariae and their adults belonging to the genus *Azygia* Looss, separating them from all the other cercariae with a tail of cystocercous chracter, but he mentioned at the same time that the Order Strigeatoidea in that case is a combination of two well separated parts of the system which do not appear to have a close relationship with one another. Wesenberg-Lund (1934) agreed with Szidat in his assignment of the Furcocystocercous cercariae to the Furocercariae and the Strigeatoidea saying that the excretory system appears to be constructed on the same plan as in the former and that Sewell is probably right in supposing that the cystocercous character of the tail may be merely an example of convergence. He considered it most correct to regard them as a special group of Furcocercariae under the name of Cystocercaria, and divided the rest of the old group of Lühe into *Macrocercaria* as maintained by Lühe and the remaining under the name of Sinitizin Crystomorpha which is indentical with the group Cystophora (Cystophorous cercariae).

La Rue (1957) has included all families of the Digenea which have fork-tailed cercarae in the Order Strigeatoidea, which contains according to his latest conception four suborders: Srigeata La Rue, 1926 with three superfamilies, Strigeoidea Railliet, 1919, Clinostomatoidea Dollfus, 1931, and Schistosomatoidea Stiles and Hassal, 1926; the suborder Azygiata La Rue, 1957 with the superfamilies Azygioidea Skrj. and Gusch., 1956 and Transversotrematoidea La Rue, 1957; the suborder Cyclocoelata La Rue, 1957 with the superfamily Cylocoeloidea Nicoll, 1934: and suborder Brachylaimata La Rue, 1957 with the superfamilies Brachylaimoidea Allisson, 1943, Fellodistomatoidea La Rue, 1957 and Bueephaloidea La Rue, 1926.

[151]

In our discussion on the recent (1957) classification of the Digenea by La Rue, we have pointed out that the cystocercous character of the cercariae is probably monophyletic and the fucocercous character polyphyletic. We excluded the sub-order *Azygiata* La Rue, 1957 with the superfamily Azygioidea Skrj. and Gejanskaja, 1956 from the Strigeatoidea La Rue and raised it to the order Azygiatoidea Mehra, 1957 and have created a new suborder Transversotremata for the superfamily Trans-versotrematoidea La Rue, 1957 to be included under this order along with suborder Azygiata. The order Azygiatoidea is characterised by the furcocystocercous cercariae developing in rediae with flame cell groups in the tail ; miracidia with one pair of flame cells ; protonephridia stenostomate : usually progenetic ; two host life-cycle ; genital opening in the adult near anterior end in front of ventral sucker or anterior to oral sucker; parasitic in fishes. This order is closely related to the order Hemiuratoidea Mehra, 1957 characterised by its highly specialized cystophorous or Hemiurid cercarae developing in rediae with caudal excretory vessels and flame cell groups in the tail ; protonephridia stenostomate, main collecting vessels fused anteri-orly ; miracidia non-ciliate, but with spinose anterior tip ; second intermediate host a copepod ; genital opening in front of ventral sucker near anterior end. This order shows resemblance to the Azyigatoidea in fundamental features of adult morphology, such as smooth cuticle, presence of genital pore in front of acetabulum near oral sucker or pharynx, excretory bladder tubular or y-shaped. It is also progenetic and its cystophorous cercariae somewhat resemble Furcocystocercous cercariae of Trans-versotremata n. so., which have a tail stem bearing a pair of anterior lateral arms. Soparkar (1924) hinted that these anterior lateral projections of the tail may be considered to represent a lateral projection from the proximal end of the tail of the cercariae of the Cystophorous group. Rothschild (1938) also mentioned that the furcocystocercous cercariae of the Azygiidae Odhner which encyst by withdrawing into their own tails represent a first stage in the evolution of the cystophorous cercariae.

C. pekinensis Faust, 1921 probably represents a primitive type of furcocystocer-cous cercariae. Szidat (1932) thought that *C. stephenocauda* Faust, 1921 and *C. pekinensis* do not belong to the Furocystocercous group. Miller (1936), however, included besides the seven furcocystocercous cercariae dealt with by Szidat, four more cercariae in this group, namely *C. pekinensis* Faust, *C. stephenocauda* Faust, *C. melanophora* Smith, 1932 and *C. hodgesiana* Smith, 1932.

Faust mentions that "the movement of *C. pekinensis* is characteristically that of the cystocercous larvae, namely, backward instead of forward. The furci are distictly flapper like". He says that *C. pekinensis* bears a striking resemblace to *C. brookoveri* Faust, 1918. It has a stenostomate type of protonephridial system. Its sporocyst with pharyngeal sphincter at anterior end but without a gut resembles closely the redia of *C. mirabilis* and *C. splendens* described by Szidat (1932) and the sporocyst of *C. brookoveri* according to Faust. The encystment of *C. pekinensis* was not observed but he says that "it seems probable that it passes directly into the subsequent host without a latent period." According to him though the tail becomes differentiated from the body of the developing cercariae soon after the germ ball stage, the furci appear late showing that in its development it fundamentally differs from the furcocercaria of the Strigeatoidea. This shows that the furocystocercous cercaria has been evolved along another line of ancestory than that of the latter.

The Macrocercous and Rhopalocercous cercariae constituting a natural group though distinctly separate from the Cystofurocercous Cercariae (Cystocercous

cercariae of Miller = Mirabilis group of Sewell) = Cystocercaria of Wesenberg
Lund) and Cystophorous cercariae as defined by Sinitsin (1911) = (Appendiculata
group of Sewell) on acconut of fundamental morphlogical differences and different
life histories besides their resemblance in the cystocercous character of their tail
present similarity in general morphological features of the adult into which they
develop, and this shows that these three groups have a close natural affinity though
they differ so much as to deserve the rank of separate orders in the taxonomic system
of the Digenea. We reiterate as mentioned before (1957 and 1958) that the evolu-
tion of the Digenea has probably taken place in three divergent lines from the
primitive digenetic ancestor, one branch giving the Orders Fasciolatoidea Szidat,
1936, Plagiorchiida La Rue, 1957 and Opisthorchiida La Rue, 1957, the other
branch giving the Order Strigatoidea La Rue, 1926 and the third line giving the
Orders Azygiatoidea Mehra, 1957, Hemiuratoidea Mehra, 1957, and the Gorgo-
derida n.o. The new superfamily Gorgoderoidea though more closely related to
the superfamilies Azygioidea Skrj. and Gusch. and Hemiuroidea Faust than to any
other distome groups, differs so much from them as to necessitate the creation of
the new Order Gorgoderida, which possess Macrocercarie and Rhopalocercariae with
chacteristic specialization in the tail and body of these larvae. The stylet in the
Macrocercariae is embeded in oral sucker dorsally to the mouth with its long axis at
right angles and dorso-ventral to the body and pointed end directed upward unlike
that of the Xiphidiocercariae in which it lies parallel to the body length with the
pointed end directed forwards. Unlike that of the latter it is also complicated
bearing several spinose points. The cercarial stylet, therefore, which is an adaptive
organ differs remarkably in the Macrocercariae and Xiphidiocercariae showing that
these two groups have no close affinity with one another. No stylet is present in the
Rhopalocercariae which as they encyst within the chambers of their own transformed
tails do not need a stylet, whereas in the case of stylet bearing macrocercariae a
second intermediate host is required for encystment. The non-stylet *C. mitocerca*
Miller, 1936, however, does not encyst within its macrocercous tail. The absence of
stylet in the microcercaria of *Phyllodistomum folium* is attributed to the fact that
it has no free living period as it encysts and becomes a metacarcaria within the
sporocyst in which it has developed. The sporocysts with the encysted metacercariae
emerge from the clam and are eaten by the definitive fish host.

Fischthal (1951) who has traced the evolution of the cercariae in the Gorgo-
deriidae thinks that these cercariae originated and first occurred in the hosts of the
family Unionidae, later in the Sphaeriidae and finally in the Dreissenidae and that
the cercariae of the two latter host families are closely related to each other. These
assumptions are primarily based on the origin and relationship of the clam hosts.
The rhopalocercariae which are parasitic in the Unionidae and have their daughter
sporocysts occurring in the visceral mass of their hosts and are without stylets are
probably primitive and are evolved from the ancestor along one line. The macro-
cercariae which with the exception of *C. mitocerca* Miller have their hosts belonging
to the Sphaeriidae with the daughter sporocysts occurring in their gills and possess
stylets arose later along another divergent line and in these encystment occurs within
second intermediate host. The macrocercous *C. microcerca* which has its host a clam
of the family Unionidae and in which a stylet is absent and probably requires a
second intermediate host connects the Macrocercariae and the Rhopalocercariae. It
stands also near the divergence of the former and the microcercaria of *C. phyllodist-
mum folium* Sinitzin (1901) as originating from the common ancestor. The latter
which possesses a degenerate stumpy tail has several features common to the rhopalo-
cercariae such as absence of stylet, complete differentiation of all parts of the
reproductive system, presence of an inactive tail and the lack of necessity for an

[153]

intermediate host for encystment. Some of the macrocercariae also have their reproductive system differentiated into all the component parts and also have an inactive tail.

The group of macrocercariae to which Krull in 1936 added *Cercaria Gorgodera ampliclava*, Steelman added *C. raicauda* in 1938 belonging to the "Gorgoderina" group and *C. coeolcerca* in 1939 belonging to the "Gorgodera" group, Goodchild in 1939 added in two separate papers, *C. doncerca* and *C. conica* which are quite similar but distinct from *C. coleocerca*, Rankin (1939) added *Cercaria Gogoderina attenuata*, Goodchild (1939) *C. phllodistomum solidum* and Baker (1943) *C. steelmani* is large. consisting of thirteen different species, of which *C. Gorgoderina attenuata* Rankin and *C. Gorgodera amplicava* Krull are the most primitive of all the macrocercariae and *C. mitocerca* Miller stands intermediate between the latter and the rhopalocercariae quite.close to the probable ancestor of the latter. These with the six species of the rhopalocercariae *C. duplicata* von Baer, 1827, *C. micromyae* Fischthal, .951, *C. catatonki* Fischthal, 1951, *C. honeyi* Fischthal, 1951, *C. pyriformis* Fischthal 1951, and *C. filicauda* Fischthal, 1951 and Microcercaria *C. Phyllodistomum folium* Sinitzin, 1901 form a large natural group belonging to the family Gorgoderidae of the new super-family Gorgoderoidea included in the new order Gorgoderida. The specialization undergone in the structure of the tail by the macrocercariae and rhopalocercariae has so effected the development of the excretory system in the tail that the growth of the developing tail from the tissue between the excretory pores so shifts the position of the latter that the lower portion of the primary tubules becomes bent and they come to open laterally at about the junction of the body with the developing tail. Thiry (1860) described a caudal excretory tube in *C. macrocerca*. According to Wesnberg-Lund (1934) in *C. vitellilobae* Ssin. and *C. Gregodereae Pagenstecheri* Ssin. the excretory bladder gives off posteriorly a tube which passes through the entire tail, but no opening was observed. Vickers (1941) refers to a group of small non-muscular cells situated between the longitudinal muscle bands and the base of the tail from which a strand consisting of a chain of small elongated cells with oval nuclei runs down the centre of the tail to its extreme tip. This chain of cells he thinks is referred to as an excretory canal by Thiry (1860) and Weswnberg-Lund (1934). He says "it is certainly not a canal." Fischthal also did not find a caudal excretory canal in Rhopalocercariae. The latter author from the method of the development of the excretory system considers it impossible that a caudal excretory canal can be present. So it is obvious that the special mode of development of the tail and its structural modification have secondarily entailed the loss of caudal excretory canal in these cercariae, and its absence, therefore, should not be considered as an objection to the relationship of the superfamily Gorgoderoidea Mehra 1958 of the order Gorgoderida Mehra, 1958 with the superfamilies Azygioidea Skr. and Gusch., 1956 and Transversotrematoidea La Rue, 1957 included in the order Azygiatoidea Mehra, 1957 and the superfamily Hemiuroidea Faust, 1929 of the order Hemiuratoiidea Mehra, 1957. The order Gorgoderida stands as an offshoot near the base of the phylogenetic branch along which the Azygiatoidea have been evolved, whereas the Hemiuratoidea which are much more specialized on account of their highly modified cystophorous type of cercariae and have a copepod as a second intermediate host may be considered to have diverged off near the end of Azygiatoidea branch. That the Hemiuratoidea represent a highly modified group of Digenetic fish trematodes is also indicated by the specialization undergone by many members in their adult morphology. The adults of many Hemiurids have become modified in the posterior part of rhe body to form a tail or an abdomen "ecosoma" capable of being retraced in the anterior part or "soma" in a telescoped condition which when fully

[154]

extended is as long or longer than the "soma." Didymozoidae Poche, 1907 included in this order is also a family of highly modified fish trematodes.

The adults of the Gorgoderoidea (order Gorgoderida) are nonspinous distomes like those of the Azygioidea (order Azygiatiidea) and Hemiuroidea (order Hemiuratoidea) as a rule with well developed suckers situated near one another. The genital opening in these three orders is situated anterior to acetabulum near anterior end. Cirrus sac is absent (present only in Azygioidea). The vitellaria are also similar, small as a rule, compact, lobed, follicular or branched in tubular or thread like branches, intercaecal, overlapping caeca or extracaecal. Uterus is large and much coiled behind acetabulum. The excretory bladder is also similar generally tubular or Y-shaped, V-or U-shapped only in Bivesiculidae Yamaguti, 1939 of Azygioidea. Azygiatoidea are parasitic in the gut, generally the stomach of fishes (Teleosts and Selachii). The Hemiuratoidea are parasitic as a rule in the gut, usually the stomach of fishes (*Halipegus* in Amphibia). The Gorgoderida are parasitic in fishes (Selachii and Teleosts), Amphibians and Reptiles, mostly in ureters and urinary bladder of Amphibians and fishes, coelom of Selachii and in a few cases the stomach of fishes.

Looss created the subfamily Gorgoderinae in 1899 with *Gorgodera* Looss, 1899 as the type genus and the family Gorgoderidae in 1901; he also created in 1901 under this family the subfamily Anaporrhutinae including under it the genera *Anaporrhutum* Ofenheim, 1900 with *A. albidum* as the type species, *Probolitrema* Looss, 1902 with *P. richardii* (Lopez, 1899) Looss, 1902 as the type species and *Plesiochorus* Looss, 1901 with *P. cymbiformis* (Rud., 1919) Looss, 1901 as the type species. Under the subfamily Gorgoderinae he included besides *Gorgodera* Looss, 1899 the genera *Phyllodistomum* Braun, 1899, with *P. folium* (Olfers, 1816) Braun, 1899 as the type, *Catoptroides* Odhner, 1902 and *Gorgoderina* Looss, 1902 with *G. simplex* (Looss, 1899) Looss, 1902 as the type. The subfamily Anaporrhutinae Looss, 1901 contains Gorgoderids parasitic in the body cavity of the Selachii with the exception of the genus *Plesiochorus* Lss., which is parasitic in the urinary bladder of marine Chelonia. Odhner (1911) suggested that the genus *Plesiochorus* Lss. should be separated from the Anaporrhutinae and should constitute a new subfamily. Yamaguti (1958) created four new subfamilies under the Gorgoderidae, Phyllodistominae—for the genera *Phyllodistomum* Braun, 1899 and *Gargotrema* Dayal, 1938, Probolitreminae for the genus *Probolitrema* Looss, 1902, Xystretinae for the genus *Xystretum* Linton, 1910 and Plesiochrinae for the genera *Plesiochorus* Looss, 1901 and *Bicornuata* Pearse, 1949. Of these subfamilies created by Yamaguti only Plesiochorinae is acceptable. This subfamily was created by me in February, 1958 as will be seen from the Proceedings of Abstracts of Papers of the 28th Annual session of the Nat. Acad. Sci. India, February 6-8, 1958. The subfamily Plesiochorinae Mehra 1959 is characterised by the presence of pharynx and two simple but deeply lobed or branched symmetrical testes, genital pore immediately in front of acetabulum, vitellaria lobate and symmetrical apart from each other just behind acetabulum in front of testes, and parasitic in the urinary bladder or gall bladder of Chelonia. The subfamily Anaporrhutinae Lss. after the separation of *Plesiochorus* from it comprises large fleshy exclusively body cavity distome parasites of Selachii with testes as a rule divided into a large number of follicles situated mostly external to intestinal caeca and vitellaria quite apart and at some distance from each other intercaecal at or near the level of ovary, extracaecal only in *Probolitrema*. There is no doubt that the subfamily Probolitreminae Yamaguti for the only genus *Probolitrema* based only on the extracaecal position of the vitellaria is untenable, as this character stands in graded relationship to the position of the vitellaria in other genera of the subfamily Anaporruhtinae Lss. The vitellaria in the

genus *Anaporrhutum* though mostly intercaecal partly overlap the caeca ; in *Probolitrema* they have shifted further outside so as to occupy a completely extracaecal position. The testes which are partly intercaecal and partly extracaecal in *Anaporrhutum* have become completely extracaecal in *Probolitrema* and other genera of the Anaporrutinae Lss. The pattern of origanisation and the habitat of *Probolitrema* conforms so much to that of the other genera of the latter subfamily that this genus cannot be separated from it. The subfamily Phyllodistominae Yamaguti created on the basis of the character of the shape of the body resembles much the subfamily Gorgoderinae Lss. in the morphological pattern and totality of its organisation. Luhe (1909) in his key of the genera of the Gorgoderinae Looss distinguished *Gorgoderina* Looss and *Gorgodera* Looss from *Phyllodistomum* Braun and *Catoptroides* Odhner by the body shape-hinder body narrow in the former two genera and the hinder body broadened with two simple testes in the latter two genera. Pande (1937) who combined *Phyllodistomum* and *Gorgoderina* into one genus drew attention to the opinions of Osborn (1903) and Sinitzin (1905) about the identity of these two genera. He also quoted Cort (1912) who thought "*G. transulucida* and *P. americamum* seemed almost to constitute a transition between these two genera". Kaw (1950) supported Pande in combining *Phyllodistomum* with *Gorgoderina* saying "the shape and external form is a variable character and cannot alone be considered of generic importance unless accompanied by other constant and important modifications of the internal organs". *Phyll.distomum almorai* Pande with an elongated somewhat flattened body and wider post-acetabular region represents in our openion another transitional form between *Phyllodistomum* and *Gorgoderina*. So when it is difficult to maintain even the individuality of the two genera, the subfamily Phyllodistominae cannot be maintained and therefore must be dropped. The distinction of the shape of body cannot indicate that constancy of a morphological character on which a subfamily should be based. According to Byrd, Venard and Reiber (1940) differences in the excretory system of *Phyllodistomum loronzi* (Lown, 1935) and *Gorgoderina tanneri* Olsen, 1937 are so well marked as to justify the validity of these genera. These authors mention that the species investigated by them are differentiated into two groups 1. *Gorgoderina-Catoprioides* group in which the common collecting tubules are long, reaching the bifurcation of the caeca before giving rist to anterior and posterior collecting tubules, 2. *Phyllodistomum-Gorgoderina* group in which the common collecting tubules are short giving rise to the anterior and posterior collecting tubules behind the level of ventral sucker. Besides the common collecting tubules emerge from the bladder just behind its anterior termination in *Gorgodera*, *Phyllodistomum* and *Catoptroides* while the bladder in *Gorgoderina* gives rise to two short dilated cornua from the ends of which the common collecting tubules arise. In view of these findings it appears that in the present state of our knowledge the validity of the genera *Phyolldistomum* and *Gorgoderina* should be maintained. Dobin Jr. (1957) giving account of *Gorgoderina parvicava* Travassos, 1922 has shown that the genus *Gorgoderina* Lss. parasitic in the Batrachia is a valid genus ; we are in agreement with this. *Phyllodistomum* though it is predominantly a genus of fish distomes, is parasitic in Amphibians also, but *Gorgoderina* is reported only from Amphibians.

There is, however, unanimity of opinion (Lewis, 1935), (Lynch, 1936), (Bhalerao, 1937) and (Yamaguti, 1950 and 1958) about the synonimity of the genera *Phyllodistomum* and *Catoptroides*. Kaw (1950) was right in doubting the validity of *Phyllochorus* Dayal, 1938. This genus as held by Yamaguti (1953 and 1958) is identical and synonymous to *Phyllodistomum*. We also agree with Yamaguti (1958) in considering Plesiodistomum Dayal, 1949 as synonynous to *Phyllodistomum*. The shape of the body, position of the vitellaria close to each other just behind acetabulum and

the general topography which is typical of the latter genus warrant it. The presence of pharynx in *P. (Plesiodistomum) callichrous* (Dayal, 1949) Yamaguti, 1958 which according to Dayal connects *Phyilodistomum* with the Anaporrhutinae Lss. may be considered as an example of reversion.
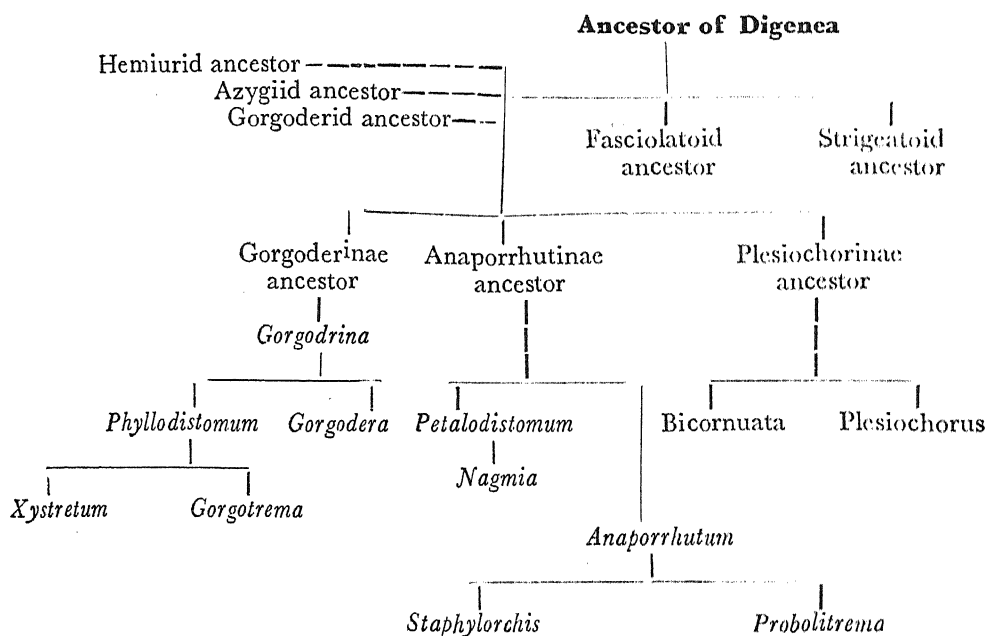
The subfamily Xystretinae Yamaguti differs from *Phyllodistomum* only in the posterior union of the intestinal caeca. The latter have become united posteriorly in *Xystretum* due to specialisation and this should not be considered as a basis of subfamily distinction. The close similarity in organisation and habitat in the urinary bladder of fishes of *Xystretum* and *Phyllodistomum* shows their close relationship and hence their inclusion in the same subfamily, therefore, the subfamily Xystretinae must be dropped. The purpose of a natural system of classification is not served if subfamilies are created on minor or rather generic characters without taking into account the similarity of organisation pattern of the genera included in them signifying close relationship.

There is no doubt that the ancestor of the family Gorgoderidae was an elongated distome probably parasite in fishes like Azgiids and primitive Hemiurids. It possessed a pharynx and two simple testes. The genera *Gorgoderina* Looss and *Gorgodera* resemble the ancestor in the shape of their body but they differ in lacking the pharynx. It appears that the trend in the evolution of most genera of the family was generally towards flattening and broadening of the body from about or behind the acetabulum and this has reached almost its culminating point in the genera of the subfamily Anaporrhutinae Lss. The genera *Bicornuata* Pears, 1949 and *Plesiochorus* Looss, 1902, which have a somewhat broadened body, uniformly broadened in *Bicornuata* and broadened behind acetabulum in *Plesiochorus* of the subfamily Plesiochorinae stand close to the ancestor. The genus *Phyllodistomum* which we include in the subfamily Gorgoderinae Lss. comprising a large number of species with broadened foliate post-acetabular hind body sharply set off from the pre-acetabular neck region with two simple testes is, as discussed already, closely related to *Gorgoderina* Lss. and has been evolved from it through the same common ancestor. From near the ancestor of *Bicornuata* Pears and *Plesiochorus* Looss have been evolved along one line the genera of the subfamily Anaporrhutinae Looss which possess the pharynx, and along another line the genera of the subfamily Gorgoderinae Looss which lack it. The innate tendency of the two primitive testes of the ancestor, entire in *Gorgoderina* and as a rule lobate in *Phyllodistomum* towards splitting up into follicles has resulted in their subdivision into a number of follicles arranged in two longitudinal rows in *Gorgodera* Looss as derived from *Gorgoderina* Looss and into a large number of follicles irregularly scattered in *Gorgotrema* Dayal, 1938 as derived from *Phyllodistomum* Braun. There is no doubt that *Gargotrema* Dayal is closely related to the latter genus on account of similarity in the shape of its body, absence of pharynx, in the position and shape of the vitellaria close to one another just behind acetabulum, but it is specialised or more advanced in possessing lateral branches of the excretory bladder and large number of testes follicles. In the subfamily Anaporrutinae Lss. the testes are divided into numerous small follicles except in *Petalodiostmum* Johnston, 1914 in which they are deeply incized or divided into several large pieces showing an incipient stage of their subdivision into follicles. They are extra-caecal except in *Anaporrhutum* in which they are partly internal and partly external to intestinal caeca ; they are intercaecal in the Gorgoderinae occupying a primitive position. The testes in the Plesiochorinae are deeply lobed or branched and are partly inter-caecal and partly extra-caecal showing an intermediate condition between that of the ancestor and of the Anaporrhutinae.

[157]

The Anaporrhutinae Looss are large sized Gorrgoderidae with distinctly broad, flattened, petaloid, almost oval or rounded body ; muscular pharynx present, caeca usually sinuous or strongly winding with outgrowths only in *Petalodistomum* and *Nagmia*. Genital pore immediately post-bifurcal or immediately pre-bifurcal. Receptaculum seminis large ; Laurer's canal absent ; metraterm long, tubular. Primitive testes divided into several pieces or follicles, lateral outside caeca, sometime overreaching them and partly inter-caecal *(Anaporrhutum)*. Vitellaria apart from each other, lobed, dendriric or branched consisting of tubular acini, inter-caecal at or near level of ovary, extra-caecal and pre-testicular in *Probolitrema*. Parasitic in body cavity of Salachii (sharks and rays).

The genus *Anaporrhutum* Ofenheim, 1900 with wide simple caeca, acetabulum near oral sucker, testes divided into symmetrical grape like bunches of follicles overlapping and outside caeca and partly inter-caecal, vitellaria dendritic just inside caeca at level of ovary and receptaculum seminis represents probably the primitive genus which comes near the ancestor of the subfamily from which have been evolved the genera *Petalodistomum* Johnston, 1914 and *Nagmia* Nagaty, 1930 along one line, *Staphylorchis* Travassos along the second and *probolitrema* along the third line. The genera *Petloadistomum* and *Nagmia* have caeca provided with outgrowths, testes deeply incised or divided into a few lobes in the former or into grape like symmetrical bunches of follicles in the latter, vitellaria inter-caecal consisting of tubular acini at level of ovary and excretory bladder with or without lateral branches. The genus *Staphylorchis* has strongly winding simple caeca, testes divided into numerous small widely diffused extracaecal follicles, vitellaria divided into tubular or thread like lobules situated inside caeca at or near level of ovary and long narrow tubular bladder without side branches.

The following schematic tree illustrates the above mentioned phylogenetic relatiohship of the genera of the family Gorgoderidae.

Ancestor of Digenea

Hemiurid ancestor

Azygiid ancestor

Gorgoderid ancestor

Fasciolatoid ancestor

Strigeatoid ancestor

Gorgoderinae ancestor

Anaporrhutinae ancestor

Plesiochorinae ancestor

Gorgodrina

Phyllodistomum *Gorgodera* *Petalodistomum* Bicornuata Plesiochorus

Xystretum *Gorgotrema* *Nagmia*

Anaporrhutum

Staphylorchis *Probolitrema*

Diagnosis : Cercariae.—Macrocercous, Rhopalocercous and Micrcercous (Microcercaria of *Phyllodistomum folium*). Adults with pre-equatorial acetabulum, genital opening median preacetabular, postbifurcal, rarely pre-bifurcal, near anterior end. Cirrus sac absent. Ovary intercaecal closely or a little behind acetabulum. Excretory bladder narrow, long, tubular. Protonephridial system mesostomate. Development of cercariae in sporocysts. Two host life cycle, usually second intermemidiate host required by stylet bearing macrocercariae for encystment.

SUPERFAMILY GORGODEROIDEA MEHRA, 1958

Diagnosis :   The same as for the order Gorgoderida Mehra, 1958.

FAMILY GORGODERIDAE LOOSS, 1901

Diagnosis :   Superfamily Gorgoderoidea. Body as a rule smooth, nan-spinous, rarely papillate. Suckers well developed. Acetabulum pre-equatorial Pharynx prsent or absent ; caeca simple, accasionally with ougrowths, or sinuaus reaching to near hinder end, occasionally united at posterior and (*Xystretum*). Genital opening median, pre-acetabular, pre-testicular, post-bifurcal, occasionally pre-bifurcal. Cirrus sac absent. Ovary intercaecal, submedian' post-acetabular, in testicular zone or a little anterior to it. Receptaculum seminis present or absent ; Laurer's canal absent or present. Testes post-acetabular, two campact, lobate or sometimes entire, or divided into several pieces or many follicles, intercaecal, overlapping caeca or extracaecal, symmetrical or asymmetrical. Vitellaria double, post-acetabular, small, compact, lobate or dendritic or branched into small tubular or thread like branches, intercaecal or occasionally extracaecal and pretesticular (*Probolitrema*). Uterus usually intercaecal, occasionally overlapping caeca or intruding into extracaecal fields. Metraterm present or absent. Excretory bladder tubular, semetimes Y-shaped. Protoneshridial system mesostomate. Excretory opening at or near posterior end. Eggs fairly large, numerous. Cercariae Macrocercous or Rhopalocercous or Microcercous (*Phyllodistomum folium*) developing in sporocysts. Two host life cycle, usually second intermediate host required by stylet bearing macrocercariae for encystment. Hosts fishes, amphibians and reptiles.

Type genus :   *Gergodera* Looss, 1899.

KEY TO SUBFAMILIES OF GORGODERIDAE

1. Pharynx absent .. Gorgoderinae

    Pharynx present .. 2

2. Testes two, not divided into pieces or follicles ; parasites of reptiles .. Plesiochorinae

    Testes devided into several pieces or follicles ; parasities of body cavity of Selachii .. Anaporrhutinae

GORGODERINAE LOOSS, 1896

Subfamily diagnosis.—Gorgoderidae : Body elodgated or divided more or less distinctly into a narrow forebody tapering anteriorly and broadened flat foliate,

oval or rounded hindbody. Pharynx absent ; oesophagus short, moderately long or long ; caeca simple, terminating blindly or united posteriorly. Testes two, compact, entire or lobed, or divided into follicles, intercaecal and situated behind vitellaria. Ovary intercaecal, submedian or median, pretesticular. Receptaculum seminis absent. Vitellaria compact, lobate, intercaecal, near each other, preovarian or in level with ovary. Genital opening postbifurcal. Parasitic in urinary bladder of fishes and amphibians.

Type genus : *Gorgodera* Looss, 1899.

KEY TO GENERA OF GORGODERINAE

1. Testes two undivided   .. (2)

    Testes divided into pieces or follicles   .. (3)

2. Body elongated cylindrical or subcylindrical   .. *Gorgoderina*

    Body divided into narrow forebody tapering anteriorly
    and broadened hindbody   .. (4)

4. Caeca united posteriorly   .. *Xystretum*

    Caeca ending blindly   .. *Phyllodistomum*

3. Body elongated, not divided into regions : testes follicles
    arranged in two longitudinal rows   .. *Gorgodera*

    Body divided into narrow forebody and expanded foliate
    hindbody; testes follicles scattered irregularly in anterior
    half of posterior portion   .. *Gorgotrema*

PLESIOCHORINAE MEHRA, 1958

Subfamily diagnosis.—Gorgoderidae : Body stout, elongated, somewhat broadend, somewhat constricted in acetabular region. Acetabulum large. Pharynx present ; oesophagus very short or absent ; caeca more or less sinuous. Testes deeply lobed or branched, symmetrical, intercaecal, and overlapping caeca, situated behind ovary and vitellaria. Ovary intercaecal, submedian, immediately behind acetabulum. Vitellaria small, lobate, symmetrical, apart from one another, immediately post-acetabular, overlaping caeca and sometimes partly extracaecal. Genital opening median immediately in front of acetabulum. Parasitic in urinary badder or gall bladder of turtles.

Type genus : *Plesiochorus* Looss, 1901.

KEY TO GENERA OF PLESIOCHORINAE

Posterior end of body and inner margin of acetabulum bicornuate
laterally ; testes branched   .. *Bicornuata*

Posterior end of body and inner margin of acetabulum not bicornu.
ate laterally ; testes lobed   .. *Plesiochorus*

[160]

Subfamily diagnosis. — Gorgoderidae : Body petaloid, oval or almost rounded, large, more or less pointed anteriorly. Acetabulum pre-equatorial, usually near anterior end. Pharynx present; oesophagus short, very short or absent ; caeca usually sinuous or strongly winding, occasionally almost straight, simple or provided with outgrowths. Testes lateral, divided into a few large lobed pieces or into numerous follicles, extracaecal, sometimes overlapping caeca and partly intercaecal. Ovary small median or submedian, post-acetabnlar between two vitellaria. Receptaculum seminis large ; Lauter's canal absent. Metraterm well developed and tubular. Vitellaria post-acetabular, quite apart and at some distance from one another, deeply lobed, dendritic or branched into small tubular or thread like acini, intercaecal, extracaecal and pretesticular in *Probolitrema*. Excretory bladder tubular with or withou lateral branches. Parasitic in body cavity of Selachii.

Type genus : *Anaporrhutum* Ofenheim, 1900.

KEY TO GENERRA OF ANAPORRHUTINAE

1. Vitellaria extracaecal .. *Probolitrema*

   Vitellaria intercaecal .. 2

2. Intestinal caeca with ougrowths .. 3

   Intestinal caeca without outgrowths .. 4

3. Testes divided into a few large lobed pieces ; vitellaria mutilobulate .. *Petalodistomum*

   Testes divided into many follicles forming grape like bunches ; vitellaria consisting of tubular acini .. *Nagmia*

4. Testes divided into numerous widely diffused small follicles outside caeca ; caeca strongly winding .. *Staphylorchis*

   Testes divided into grape like bunches of numerous small follicles partly extracaecal and partly intercaecal; caeca not winding .. *Anaporrhutum*

REFERENCES

Baker, J. R., 1943. *Cercaria steelmani*, a new macrocercus form. *Tr. Am. Micr. Soc.* **62** : 280-285.

Baylis, H. A., 1927. Notes on three little-known Trematodes. *Ann. Mag. Nat. Hist. Ser.* 9, XIX : 627-634.

Bhalerao, G. D., 1907. Studies on the Helminths of India. Trematoda IV. *J. Helminth.* **15** : 97-124.

Byrd, E. E., Venard, C. E., and Reiber, R. J., 1940. The Excretory System in Trematoda. 1. Studies on the Excretory System in [the] Trematode Subfamily Gorgoderinae Looss. 1899. *J. Parasitol.* 26 : 407-420.

Chaudhuri, B. L. 1916. Fauna of the Chilka Lake. Fish. Pt. 1. *Memoirs of the Ind. Mus.*, **5** (4) : 403-39.

Cort, W. W., 1912. North American frog bladder flukes. *Tr. Am. Micr. Soc.* **31** (3) : 151-166.

————1914. Larval trematodes from North American fresh-water snails. *J. Parasitol.* **1** : 65-84.

Cort, W. W. and Nichols, E., 1920. A new cystophorous cercaria from California. *J. Parasitol.* **7** : 8-15.

Crusz, H., 1957. A New Anaporrhutine Trematode, *Staphylorchis parisi* sp. nov. from the shark *Scoliodon walleehini. Ceyl. J. Sci. Sect. B. Zoology,* **25** (3) : 193-195.

Dawes, B., 1946. The Trematoda with special reference to British and other European Forms. Cambridge Univ. Press : 1-644.

————1947. The Trematoda of British Fishes. *Ray Society London.* 1-364.

Dayal, J., 1938. A New Trematode, *Phyllochorus macronius* N. Gen., N. Sp. belonging to the family Gorgoderidae Looss (1901), from the body cavity of a fresh water fish, *Macrones tengara. Proc. Ind. Acad. Sci.* **7** (3) : 138-142.

————1938 A new trematode, *Gargotrema barbius* n.g., n.sp., from a freshwater fish, *Barbus sarana. Proc. Nat. Acad. Sci.* **8** (8) : 63-67.

————1949. Trematode Parasite of Indian Fishes, Part II. *Ind. J. Helminth.* **1** (2) : 93-120.

Dobbin, J. E. Jr., 1957. Fauna Helmintologica De Batraquios De Pernambuco, Brasil. 1. Trematoda. *Anais Da Socied. Biolog. Pernambuco.* XV (1) : 29-61

Dollfus, R. P., 1937. Les Trematodes Digena Des Sclaciens (Plagiostomes) Catalogue Par Hotes ; Distribution Geographique, *Ann. Parasitol. Hum. et. Compar.* XV (3) ; 57-73, 164-176, 259-281.

Fischthal, J. H., 1942. Three New Species of *Phyllodistomum* (Trematoda : Gorgoderidae) From Michigan Fishes. *J. Parasitol.* **28** (4) : 269-275.

————(1951). Rhopalocercariae in the Trematode Subfamily Gorgoderinae. in *Amer. Midl. Nat.* **46** (2) : 395-443.

Goodchild, C. G., 1939a. *Cercaria donecerca* n. sp. (gorgoderid cercaria) from *Musculium partumeium* (Say). *J. Parasitol.* **25** (2) : 133-136.

————1939b. *Cercaria conica* n. sp. from the clam *isidium abditum* Haldeman. *Tr. Am. Micr. Soc.* **58** : 179-184.

————1943. The life-history of *Phyllodistomum solidum* Rankin, 1937, with observations on the morphology, development and taxonomy of the Gorgoderidae (Tremoda). *Biol. Bull.* **84** : 59-86.

Hussey, K. L., 1941. Comparative Embryological Devolopment of the Excretory System in Digenetic Trematodes. *Tr. Am. Micr. Soc.* **69** (2) : 171-210.

Ingles, L. G. and Langston, C. 1933 A new species of bladder fluke from California frogs. *Tr. Am. Micr. Soc.* **52** (3) : 243-246.

Johnston, S. J., 1913. On some Queensland Trematodes, with Anatomical observations and Descriptions of New Species and Genera. *Quart. Journ. Micr. Sc.* **59** (3) : 361-400.

Jonston, T. H. 1934. New trematodes from South Australian Elasmobranchs. *Austr. J. Exp. Biol. and Med. Sc.* **12** (1) : 25-32.

Joyeux, C. E. and Baer, J. G., 1934. Note sur une nouvelle espèce de trématode, *Gorgoderina capensis* n. sp. *Rev. Suisse. Zool.* 41 (9) ; 197-201.

Kaw, B. L. 1950. Studies in Helminthology : Helminth Parasites of Kashmir. Part 1. Trematoda. *Indian J. Helminth*, 2 (2) 67-126.

Krull, W. H. 1935. Studies on the life history of a frog bladder fluke, *Gorgodera amplicava* Looss, 1899. *Papers Michig. Acad. Sci. Arts. and Letters* 20 : 697-710.

Lewis, F. J. 1953. The Trematode Genus *Phyllodistomum* Braun. *Tr. Am. Micr. Soc.* 54 (2) : 103-117.

Linton, E. 1910. Helminth Fauna of the Dry Tortugas II. Trematodes. *Carn. Inst. Wash. Publ. No. 133* : 1-93.

Looss, A. 1899. Weitere Beitrage zur kenntnis der Trematoden-fauna Aegyptens, zugleich Versuch einer naturlichen Gliederung des Genus *Distomum* Retzius. *Zool. Jahrb. Syst.* 12 ; 521-784.

———1902. Ueber neue und Bekannte Trematoden aus Seechildkroten. *Zool. Jahrb. Syst.* 16 (3-6), 411-894.

Lühe, M. 1906. Report on the Trematode parasites from Marine fishes of Ceylon. *Report to the Government of Ceylon on the Pearl Oyster Fisheries of the Gulf of Mannar.* Part 5 : 97-108.

———1909. Parasitische Plattwurmer. 1. Trematoden. *Susswasserfauna Deutschlands.* Heft 17 : 1-217.

La Rue, G. R. 1957. Prasitological Reviews. The Classification of Digenetic Trematoda : A Review and a New System. *Exper. Parassitol.* 6 (3) 306-344.

Le Zotte, L. A. Jr. 1954. Studies on the marine digenetic trematodes of Puerto Rico : the family Bivesiculidae, its biology and affinities. *J. Parasitol* 40 (2) : 148-162.

Manter, H. W. 1926. Some North American Fish trematodes. *Illin. Biol. Monogr.* 10 (2), 127-264.

Mehra, H. R. 1957. The Classification of Digenetic Trematoda. *Nat. Acad. Sci. Ind. Annual Number Biolog. Sec. Abstract.* : 71-72.

———1958. On a New Trematode *Staphylorchis scoliodonii* n. sp. (Family Gorgoderidae Looss, 1901 : Subfamily Anaporrhutinae Looss, 1901) with Phylogenetic Discussion and Classification of the Gorgoderidae. *Nat. Acad. Sci. Ind. Annual Number. Sec. Biol. Sci. Abstract* : 87-88.

Miller, E. L. 1936. Studies on North American Cercariae. *Illin. Biol. Monogr.* 14 (2) : 1-125.

Nagaty, H. F. 1930. A new Anaporrhutine Trematode Genus and Species *Nagmia yorkei*, with a Review of the Classification of the Subfamily. *Annals of Trop. Med. Parasit* 24 (1) : 97-108.

Nicoll, W. 1934. Vermes Section in *Zool. Rec.* : 96-98.

Odhner, T. 1911. Nordostafrikanische Trematoden grösstenteils von Weissen Nil. *Res. Swed. Zool. Exp. Egypt and White Nile.* IV. Upsala : 1-170.

Odlaug, T. O. 1937. Notes on the development of *Gorgodera amplicava* in the final host. *Biol. Bull.* 72 (1) : 80-87.

Ofenheim, E. Von. 1900. Über eine neue Distomidengattung. *Inaug. Diss.* 1-42 and *Zeitschr Naturw.* 73 : 145-186.

Olsen, O. W. 1937. A new Species of Bladder Fluke *Gorgoderina tanneri* (Gorgoderidae : Trematoda) from *Rana pretiosa*. *J. Parasitol.* 23 (5) : 499-503.

Pande, B. P., 1937. On the Morphology and Systematic Position of a New Bladder Fluke from an Indian Frog. *Ann. Mag. Nat. Hist.* Ser. 10. 20 : 250-256.

Rankin, J. S. (Jr.) 1939, The Life cycle of the Frog Bladder fluke *Gorgoderina attenuata* Stafford, 1902. *Amer. Midl. Nat.* 21 (2) : 476-488.

Reuss, H. 1903. Die Carcariae und Sporacyste des *Distomum duplicatun* Baer. *Ztschr. Wissench.* Zool. 74 458-477.

Rothschild, M. 1938. *Cercaria sinitzini* N. Sp., A Cystophorus Cercaria from *Peringia Ulvae* (Pennant 1777). *Novit. Zool.* 41 : 42-57.

Samuel, M., 1952. A new species of Coelomic Trematode of the Genus *Staphylorchis* from the Tiger shark *Galeorcerdo tigrinus* from Indian Waters. *Proc. Ind. Acad. Sci.* Sect. B. 36 (4) : 169-179.

Sewell, R. B. S., 1922. Cercariae indicae. *Ind. J. Med. Res.* **10**. Supplement : 1-370.

Sinitzin, D. T., 1901. Einige Beobachtungen über die Entwicklungs-geschichte von *Distomum folium* Olf. *Zool. Anz.* **24** : 689-694.

———1905. Distomes des poisson et grenouilles des environs de Varsovie. Materiaux pour l'histoire naturell des trématodes. *Mem. Soc. Nat. V arsovie Biol.* **15** : 1-210.

———1906. Beitrage zur Naturgeschichte der Trematoden : Die Distomen der Fische und Fröshe der Umgebung von Warschau (Original in Russian). *Zool. Ctbl.* **13** (21) : 681-689.

———1907. Observations sur les metamorphoses des trematodes. *Arch. Zool. Exp. et Gen.* s. 4. 7 (2) : 21-37.

———1907. Life history of trematodes of fishes and amphibians. *J. Roy. Micr. Soc.* **1** : 46-47.

Southwell, T., 1913. Notes from the Bengal Fisheries Laboratory, Indian Museum, No. I., On some Trematodes and Cestode parasites from fish. *Rec. Ind. Mus.* Calcutta. IX : 79-103.

Srivastava, H. D., 1938. A new Gorgoderid Trematode from Urinary Bladder of an Indian Migratory Fish, *Belone strongylura*. *Ind. J. Vet. Sc. and Anim. Husb.* **8** (4), 391-393.

Steelman, G. M., 1933. A description of *Cercaria raicauda* n. sp. *Amer. Midl. Nat.* 20 : 613-618.

———1939. A new macrocercous cercaria. *Trans. Am. Micr. Soc.* 58 : 258-263.

Stunkard, H. W., 1935. A New Trematode *Probolitrema californiense* from the coelom of the Sting Ray, *Myliobatus californicus. J. Parasitol.* 31 (5) : 359-364.

Travasses, L., 1922a. Contribuicóes para o conhecimentó da fauna helminto-logica brazileira XIV. Especies brazileiras da Familia Gorgoderidae Looss, 1901. *Brazil Med. ann.* **36** v. 1 (2) ; 17-20.

Travasses, L., 1922b. Contribuicóes para o conhecimentó da fauna helmintologica brasileria XVII. Gorgoderidae brasileiras. *Mem. Inst. Oswaldo Cruz* **15** : (1) 220-234.

Wesenberg-Lund, C. J. 1934. Contributions to the Development of the Trematoda Digenea. Part II. The Biology of the Freshwater Cercariae in Danish Freshwaters. *Mem. Acad. Roy. Sci. et Lett. Denemark.* Sec. Sci. 9 Ser. **5** : 1-223.

Walker, T., 1937. On the anatomy of *Gorgoderina vitelliloba* (Olss). : a trematode from the urinary bladder of *Rana temporara. Proc. Zool.* Soc. London 107 : 75-84.

Woolcock, V. 1 935. Digenetic Trematodes from some Australian Fishes. *Parasit.* **27**(3) : 309-331.

Wu. K., 1938. Progenesis of *Phyllodistomum lesteri* Sp. Nov. (Trematoda : Gorgoderidae) in Freshwater Schrimps. *Parasit.* **30** (1) : 4-19.

Wunder, W. 1924. Bau, Entwicklung und Funktion des Cercarienschwanzes. *Zool. Jchrb. Abt. Anat.* **46** : 303-342.

Yamaguti, S. 1953. Systema Helminthum Part 1. Digenetic Trematodes of Fishes. Published by author, Tokyo : 1-405.

———1958. Systema Helminthum Vol. 1. The Digenetic Trematodes of Vertebrates Parts I and II. Published by author : 1-1575.

ON THE SKELETAL SYSTEM OF POLISTES HEBRAEUS (FABR)

By

R. D. SAKSENA

B. R. College, Agra

INTRODUCTION

The study of Hymenopterous insects has always attracted attention of entomologists and many of the early workers studied the skeletal system in parts. Jurine and Chabbiet described the hymenopterous thorax but Latteille was the first to establish that in Hymenopterin Apocrita the first abdominal tergite has lost its relation to the abdomen and is deposited on the dorsal wall of the thorax ; Braüer (1882) and Amans (1885) corroborated Latreille's observations. Later on, Crampton, in a series of papers on the development of the thoracic sclerites in the different orders of insects, dealt with the exoskeleton of Hymenoptera in details. The study of the head of Hymenoptera dates back to 1881 when Macloskie published his work on Bees. He was followed by Schenk (1861) ; Ruhland (1888) ; Child (1894) ; Vogel (1911) ; McIndoo (1916) ; Nelson (1918) who was perhaps the first to study the abdominal sclerites of the honey bee and it was Snodgrass, who gave a detailed account of the various plates and their development. But the work on the morphology of *Polistes* is sigularly scanty ; hence it is attempted to present the detailed study of the skeletal system of *Polistes*.

MATERIAL AND METHODS

The insects were caught on wings and their skeleton was studied after killing them in cyanide bottles. Permanent preparations of different parts were made in the usual way. Hard Chitinous parts were boiled in 1% potassium hydroxide solution and were successfully stained in Ziel's Carbol Fuchsin and mounted in balsam.

Observation.—The skeletal system in *Polistes* consists of an exoskeleton of sclerites and a hard chitinous endoskeleton forming tentoria or furcae within the body. The sclerites, which form a thick chitinous shield for the body, are provided with setae thinly scattered all over except at the posterior margin of the telson, and the distal portion of the leg. The skeletal plates also bear externally various kinds of ridges, carinae, furrows, spots, pits and bands, as well as rough surfaces internally. The description of the skeletal system may be conveniently divided under three heads *viz.* the Head, Thorax and Abdomen.

The Head.—The head of Polistes is composed of six chitinous plates or sclerites *i.e.*, one clypeus, two frons, two genae and one occepital plate. In the adult, however, the sutures of these individual plates are more or less obscure so that only the clypeus is well marked.

Sutures.—The Epicranial sutures (Pl. I, Fig. 1, *Ep. Su*) is indistinct owing to the great reduction of frons. It can be seen in freshly emerged adults as a faint

[166]

line running from the scrob to the front ocellus, beyond which it becomes untraceable. The occipital suture (Pl. I, Fig. 1, *Oc. Su.*) is quite prominently visible round the posterior portion of the head, with a shallow notch at the vertex. It ends ventrally on each side of the head before the mandibular articulation, where it becomes almost indistinct. The internal cuticular ridge of this suture strengthens the cranial walls. The post occipital suture (Pl. 1, Fig. 1, *Po. Su.*) very closely surrounds the occipital foramen forming very strong *post occipital ridge* internally to which neck and prothoracic muscles are attached. This ridge is produced ventrolaterally on each side in a small process—*the occipital condyle* to which the *cervical sclerites* articulate. Two pits on either ventrolateral side of this suture mark the position of the origin of the posterior arms of the tentorium. *The Pleurostomal and hypostomal* (Pl. I, Fig. 2, *Hst.*) areas are completely fused. The *hypostomal* areas extend narrowly mesad and unite forming a hypostomal bridge which closes the occipital foramen ventrally and separates it from the gular fossa. The *ocular, ocellar* and *antennal* sutures surround the orbits of the compound eyes, the ocelli and the antennae respectively. The *subgenal suture* (Pl. I, Fig. 1 and 2 *Sg. Su.*) also exists in between the *mandibles* and the *genae*. It meets the *clypeus* anteriorly and is continued into the *geno-clypeal suture* (Pl. I, Fig. 1, *Ge. Su.*) running dorsally along the *clypeus*. The anterior arms of the tentorium arise from this place and their origin is indicated by pits externally. The *Epistomal suture* is extremely prominent in between the frons and clypeus and it owes its peculiar outline (Pl. I, Fig. 1. *E. Su.*) to the characteristic shape of the *clypeus*. It laterally meets the genoclypeal suture.

Clypeus.—The *clypeus* is more or less flat in male and anteriorly convex in the female. This is significant due to the comb-making habits of the workers. The curvature of the plate probably gives more space to the lower chamber of the buccal cavity and is of importance in the chewing of fibres into a pulp for nest-building. It is heptagonal in female and somewhat shield-shaped in male as shown in the figure (Pl. I, Fig. 7.) It occupies lower half of the head ; sparsely clothed with fine yellowish brown setae, which are relatively stouter and longer than elsewhere, specially so at the oral third, besides being denser ; slightly broader than long and nearly half as long as broad ; tentorial pits at the lateral margins ; oral border black, highly chitinised and angularly projected in the middle as to overhang the mouth ; prolonged into irregular lobes laterally, very prominent in males, in between the genae and mandibles.

Frons.—The two *frontals* (Pl. I, Fig. 1, *Fr.*) which occupy the upper portion of the head are fused together to form a median *epicranial suture*, which is indistinct except in the *interscrobal* ridge. They cover one-third of the length of the face ; as sparsely clothed with yellowish brown setae as the *clypeus* but denser along the ridge ; ventro-lateral angles produced into a triangular prolongation in female and subtriangular in male in between the eyes and the upper lateral margin of the *clypeus* at the base of these processes they surround the fourfifths of the antennal socket. In the upper third they have a subtriangular lateral process forming the sinuate ocular border. There is a fine reddish brown spiny process between the antennal socket, very prominent in males.

Genae.—The *parietals* and *genae* are closely fused into one and surround the compound eye on the ventral and posterior sides. As the eye remains far above the mandibular articulation, a large area of gena intervenes in between, and meets the lower lateral margin of the *clypeus*. It is continued further to a short distance in male, owing to which the ocular margin does not touch the clypeus. The *geno-clypeal suture* is distinct although not very long.
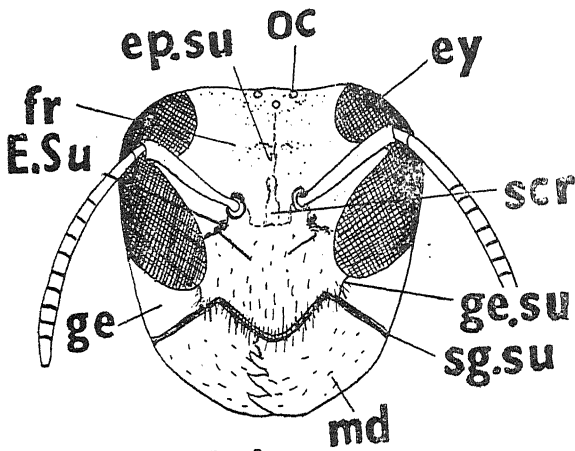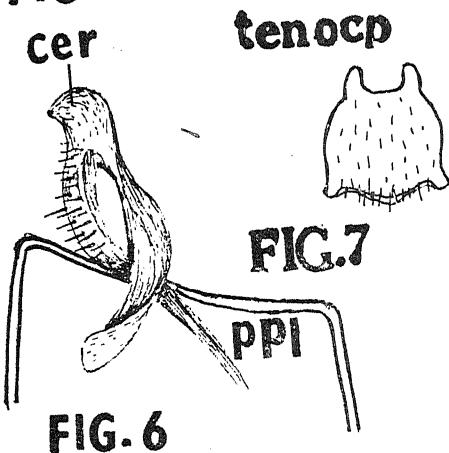
FIG.1
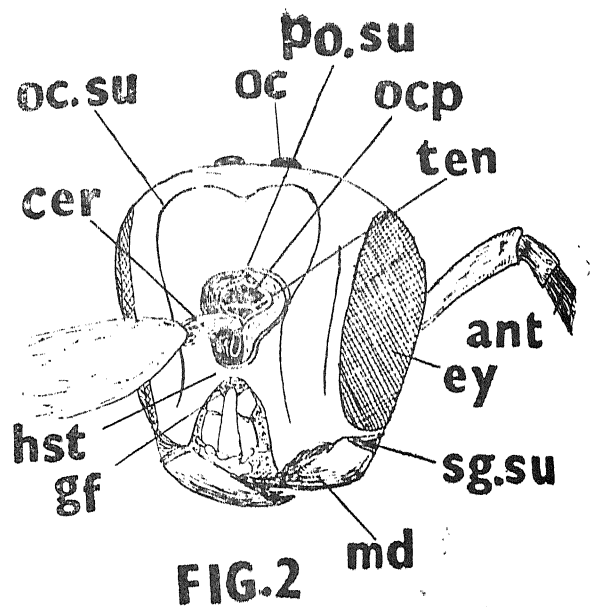
FIG.2
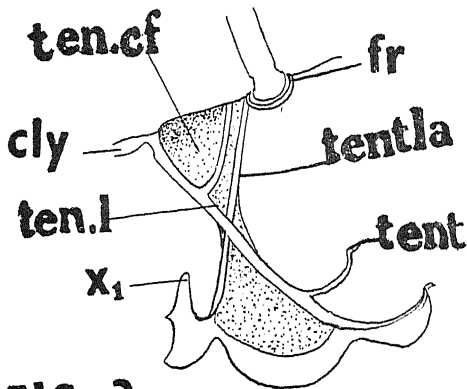
FIG. 3
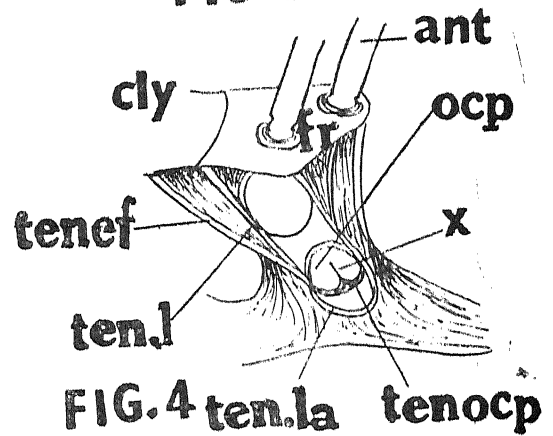
FIG. 4

FIG. 6
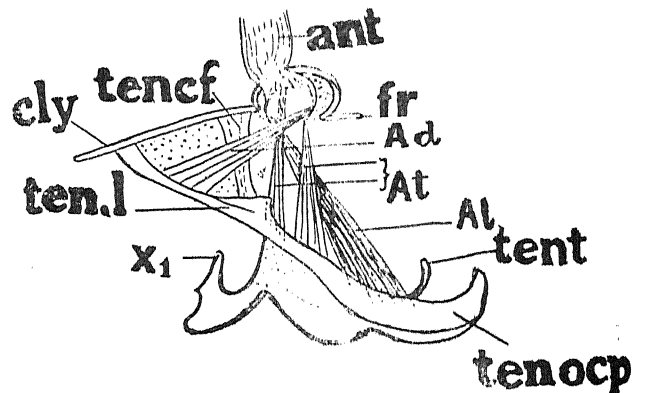
FIG.7

FIG. 5

PLATE I

Fig. 1. Front view of the head of a worker.
Fig. 2. Postero-lateral view of the head of Polistis
       with its connection to the neck.
Fig. 3. Lateral view of the tentorial pillar.

Fig. 4. Back view of the tentorium.
Fig. 5. Antennary muscles and tentorium.
Fig. 6. Attachment of cervical sclerite over propleuron.
Fig. 7. The clypeus of a male.

[168]

The *Occipital plate* (Pl. I, Fig. 2, *Oc.*) on the posterior surface of the head is a horse-shoe shaped band arching over the occipital foramen on the dorsal and lateral sides. The postgenae form the ventral boundary of the occipital foramen by means of a gular or hypostomal bridge.

The Internal Skeleton of the head.—The sutures generally mark the presence of endoskeletal ridges of cuticula. Such chitinuous ridges, beside strengthening provide for the attachment of muscles. In between the front and back walls of the cranium, there exists two large oblique chitinuous bars the *tentoria*, (Pl. I, Figs. 2, 3, 4 and 5, *ten.*) forming stout pillars. The two ends of each are broad, the anterior running laterally beneath and along the orbital margin, and the posterior forming the lateral and partly posterior boundaries of the occipital foramen, already mentioned. They act as powerful braces between the two walls of the head. Each pillar is a broad plate somewhat twisted about its middle. Its margins are specially chitinised into rod-like structures, *the arms of the tentorium.* The arm, forming its ventro-lateral margin meets the clypeus at its ventral corners and also forms the dorso-lateral border of the occipital foramen at the other end. Berlese has named it as *Tentorium longitudinale* (Pl. I, Figs. 3, 4 and 5, *Ten. l.*). The next arm, which is to be found at the dorsal margin of the pillar-like structure runs between the base of the antenna and the ventral end of the occipital foramen called *Tentorium longitu-dinal antennalis* (Pl. I, Figs. 3, 4 and 5 *Ten. la.*). It is more or less transverse. A third arm runs from the lateral margin of the clypeus and meets the other two at the point of the twist in the middle of the pillar and is termed as *clypeo-frontal branch* of the tentorium longitudinale (Pl. I, Figs. 3, 4 and 5 *Ten. cf.*).

These pillars provide important attachments to the musculature of the mouth, parts and antennae. Through the space between the two pillars the oesophagus. nerve cord and tracheal trunks pass into the cavity of the head. From the dorsal end of each pillar, a thin chitinous arm *Tentorium transversal* (*Ten, t.*) runs dorsally and meets its fellow of the other side, forming a process at the junction (Pl. I, Fig. 4, *x.*). This can be easily seen through the *occipital foramen* and ventral to this foramen is the big fossa-the gula. The walls of the occipital foramen and the gular fossa are raised into horizontal ridges *Tentorium occipital* (*Ten. Ocp.*) projecting into the cavity of the head for the suspenstion of the mouth parts. These ridges are thickened at their inner margins and a process (Figs 3 and 5 *x* 1) projects into the cavity of the head from each side. The ridges, the processes, the fossal and cranial plates with the rest of the tentoria provide attachment to the musculature of the mouth parts and antenna.

The head is articulated to the thorax by means of special lateral plates, the *cervical sclerites*, one on either side. Each plate appears in the form of a process originating from the antero-lateral margin of the prothoracic pleurite. They are very closely attached to the lateral sides of the anterior margin of the propleuron (Pl. I, Fig. 6, *P. Pl.*). From the base of each of them two long apodemal apophyses run, one along the postero-lateral side and the other ventrally inwards a short dis-tance and terminating abruptly by forming a thick rod-like structure to which important muscles from the furca are attached. The cervical sclerite (Pl. I, Figs .2 and 6 *Cer.*) is a small somewhat club-shaped plate with a knob-like fulcral point at the anterior and two apophyses at the posterior end. The knob articulates with the horizontal processes of the lateral margin of the *occipital foramen.* The body of the cervical sclerite is perforated by a foramen round which several muscles are attached, which arise on the back of the head or on the pronotum. This aperture presumably is to give a secure attachment to the tendon for the easy manipulation of the head There is a dense fringe of fine long setae round this aperture.
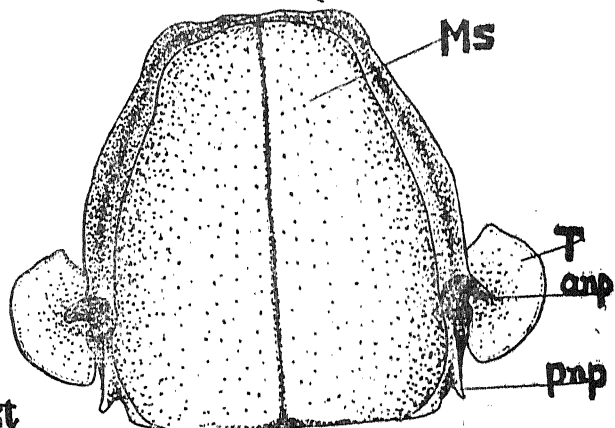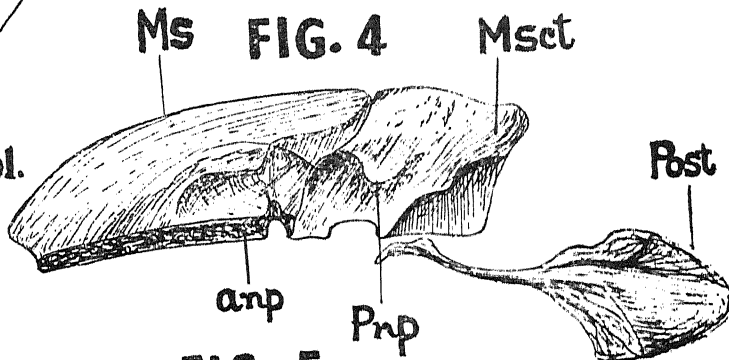
[169   ]
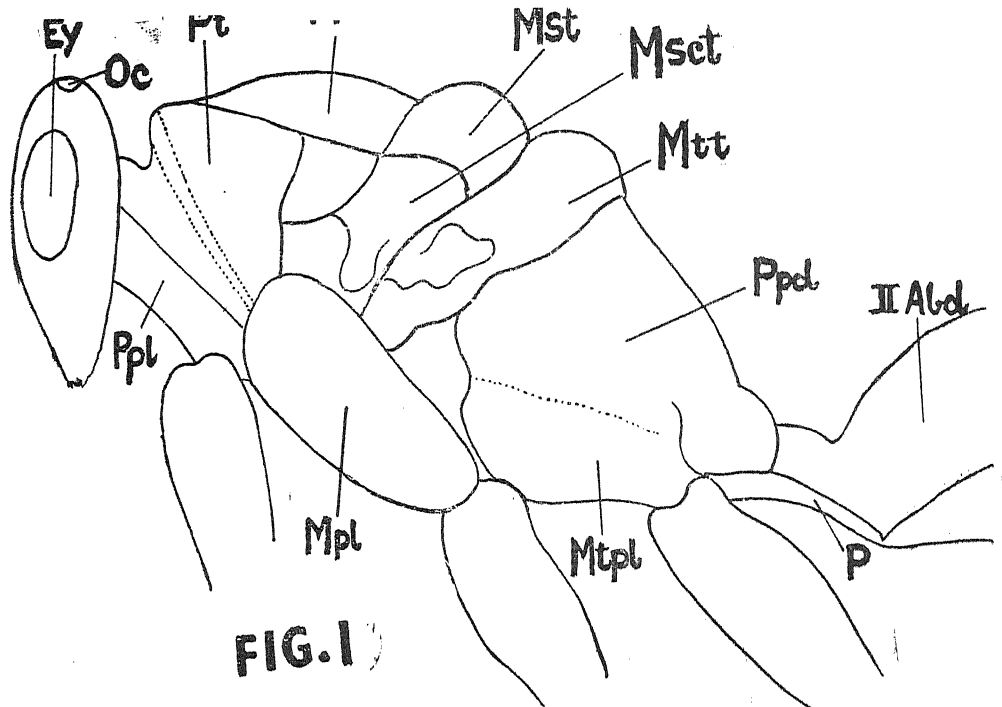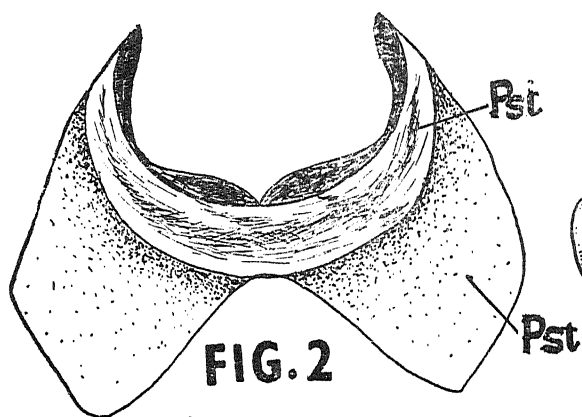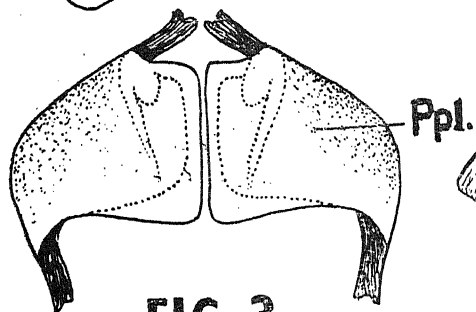
FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 5

PLATE II

Fig. 1. Lateral view of the various sclerites of the thorax.
Fig. 2. Dorsal view of the protergite.
Fig. 3. Ventral view of the propleura.

Fig. 4. Ventral view of the Mesoscutum.
Fig. 5. Lateral view of the mesothorax with its various component sclerites.
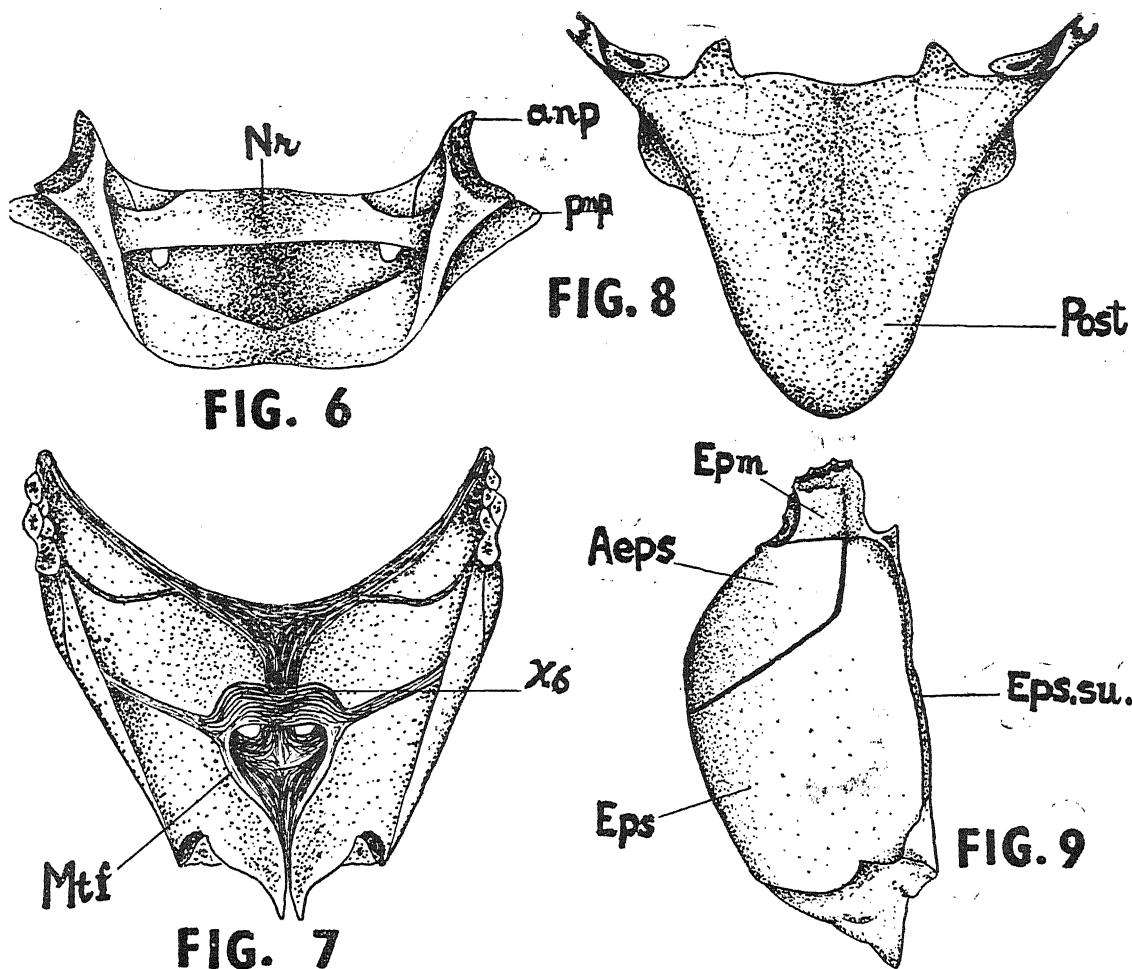
[170]

FIG. 6

FIG. 7

FIG. 8

FIG. 9

PLATE II (*continued*).

Fig. 6. Ventral view of the meso-scutellum,
Fig. 7. Dorsal view of the mesopleura after removing mesoterga showing mesofurca *in situs*.
Fig. 8. Dorsal view of the post-scutellum.

Thorax.—The thorax consists of four highly modified segments, *viz.*, the three thoracic and the first anterior-most abdominal, the propodeum (Pl. II, Fig. 1). The pro-and metathoracic portions are very narrow, while most of the thorax is occupied by the mesothorax and the propodeum. The lateral and ventral portions of the prothorax are distinctly separated off from the rest of the thorax. Each thoracic segment consists of a dorsal tergite, a pair of a lateral pleurites and a ventral sternite. Nearly all these plates are further subdivided into smaller component sclerites.

Prothorax—Since the parts of the prothorax have been more or less completely dissociated, the tergum and the pleurosternum do not appear to belong to the same segment. In fact a wide membrane separates them and there is no direct articulation in between. The prothorax, as seen from the dorsal side, occupies only the fifteenth part of the whole thoracic length but as seen laterally it appears to be about one third.

The *protergum* consists of two plates, *viz.*, an anterior *pro-scutum* and a posterior *Pro-scutellum*. The *Pro-scutum* (Pl. II, Fig. 2 *P Sc.*) is an incomplete ring set like a collar over the anterior border of the thorax. Its maximum expanse sideways is about two thirds of what it is at the thorax. The anterior margin of this plate is bounded by a thick cuticular phragma to the under and inner side of which the cervical membrane is attached. Its lateral arms taper, curve to the ventral side and terminate in an acute point forming the latero-coxal bridge of the front leg. The *pro-scutellum* (Pl. II, Fig. 2, *P St.*) is very narrow mesially rather indistinguishable from the phragma of the scutum, broadly triangulated laterad. Its postero-lateral margin is produced into a small process concave in its superior third contiguous with anterior margin of *mes-epimeron* and again concave in its inferior two thirds contiguous with the anterior margin of the *mes-anepisternum*. Only a prominent ridge of this plate is visible in the mid-dorsal line. But laterally it covers about one third of the thoracic length. In the deep notch formed by the reduction of the *Pro-scutellum* on the dorsal side is fitted the large oval anterior half of the mesothoracic scutum. Underneath the middle lobe of the posterior margin of this plate is concealed the first thoracic spiracle in the intersegmental membrane.

The *propleura* (Pl. II, Figs. 1 and 3, *Ppl.*) are very large, somewhat convex plates looking like isosceles traingles. Each plate extend ventrally and posteriorly almost meeting its fellow of the other side. It is nearly half as long as the prothorax when seen in a lateral view and half as broad as the thorax. A faint median suture divides the propleurites into an anterior and a posterior portion, which may be regarded as *pro-epimeron and pro-episternum* respectively. The anterior inner angle of the *pro-epimeron* just touches the point of attachment of the cervical sclerites and its base forms the lateral wall of the coxal fossa which is bounded by the episternum posteriorly.

The *prosternum* (Pl. III, Figs. 6 and 7, *Pstn*) consists of an anterior triagular plate which lies hidden under the propleurites just anterior to the base of the front coxae. The lateral edges of these plates are attached to the episternum ventrally. The posterior part of the prosternum lies exposed between the two front coxae in the form of two adjacent flat plates bent abruptly upwards. They bear a strong furca, (Pl. III, Fig. 7, *PF.*) which provides for muscle-attachment. Here the suppression of the sternum has partly taken place *i e*, the anterior portion has been overlapped while the posterior has not changed its position. The fore-legs are independent of the sternum and are simply connected to it by means of a thin membrane only.

[172]

Mesothorax.—It occupies the major portion of the insect's thorax and its large size is most suited to lodge the powerful mesothoracic mucles, which work both the pair of wings.

The *mesotergum* is a broad subtriangular plate rounded anteriorly, almost straight posteriorly. A transverse suture in the caudal fourth divides it into an anterior *meso-scutum* and a posterior *mesoscutellum* (Pl. II, Figs. 1-5, *Ms and Msct.*) the former is slightly over two thirds of the latter and a bit less than one third of the mesothorax which covers about two thirds of the whole thorax in length and breadth. The anterior notal wing processes of the former (*anp*) and the posterior notal-wing (*pnp*) processes of the later support the articulation of the wings. The mesoscutum forms a roof over the mesothorax and is fitted into the notch formed as a result of suppression of the proscutellum. Its posterolateral margins have prominent apodemal ridges touching over the tegulae (T). The mesoscutellum is a narrow subquadrate plate with rounded angles. Not only the external suture separates it from the mesoscutum, but the existence of a stout notal ridge situated internally further sets it apart. To the posterior margins of the *meso scutellum* articulate the thin lateral arms of a large triangularly convex plate, which is, in fact, its own posterior extension and may therefore be termed as the *Post scutellum* (Pl. II, Figs. 5 and 8, *Po. St.*). It more or less lies internally within the body cavity and is invisible from the outside except at the extreme lateral ends underneath the base of the front pair of wings. Its phragmata provides a very important place for muscle attachment. The particular position of this plate probably helps in the rapid vibrations of the wings.

The *mesopleuron* (Pl. II, Fig. 1, *Mpl.* and Fig. 9.) covers the largest area nearly one half the length laterally extending on each side between the pronotum anteriorly and the metapleuron and the coxa of the middle leg posteriorly. The uppermost part of this plate is composed of a minute sclerite, the rudimentary *epimeron* (Epm.). Its posterior margin is expanded into an irregular wide horizontal plate with processes lying below the base of the anterior pair of wings. The epimeron is continued ventro-posteriorly into a narrow strip along almost half the length of the metapleural suture. It articulates with the end of the postscutellum. From the antero-superior portion of the mesopleuron is cut off a dorsal triangular plate covering a little less than one fourth of its area—the *anepisternal* (Aeps.) plate from the main body of the sclerite which is the *episternum* (Eps.).

The *mesosternum* in wasp resembles very closely that of the honey bee. It forms the ventral region of the pleurosternal area of the mesothorax. Both the lateral episternites are continued ventrally. They fuse together by a midventral suture. A marginal band of mesosternum (Pl. III, Fig. 8, *Mb.*) is raised anteriorly into a carina while its main body is seen as a very thin line running between the mid ventral episternal suture (Pl. II, Fig. 9, *Eps. Su.*). This extremely thin portion of the mesosternum can be seen to be joining the above mentioned marginal band and can be posteriorly traced up to a wide depression of the posterior part of the sternum which projects between the meso-coxal cavities. The middle coxal process arises from the pleurosternite plates, whose posterior margins form the antecostal bridge. In the mesothorax, it is *apparent* that the sternum is almost suppressed and only its rudiments exist in the form of marginal band and a midepisternal sutural line. The greater part of its body has entered the thoracic capsule to form a very strong furca to which the powerful wing muscles are attached.

The *Metathorax* (Pl. II, Fig. 1, *Mt. t.*) is a very highly reduced narrow segment about two thirds of the mesoscutellum in length. It comprises a few sclerites,
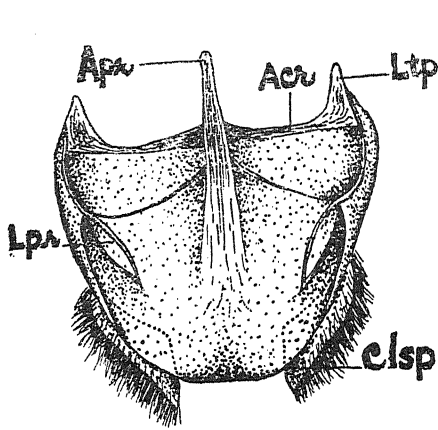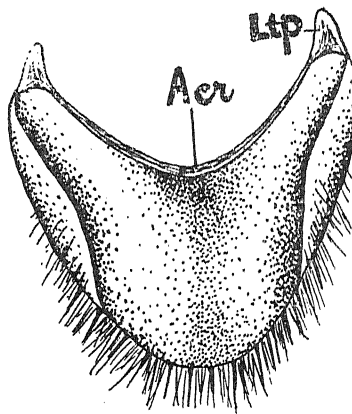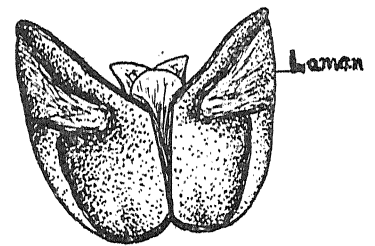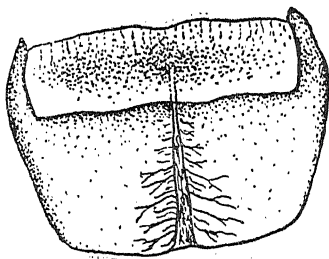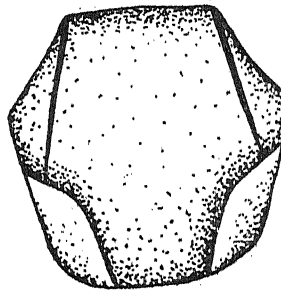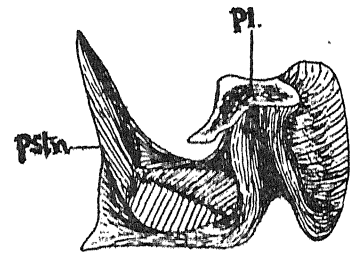
FIG. 1
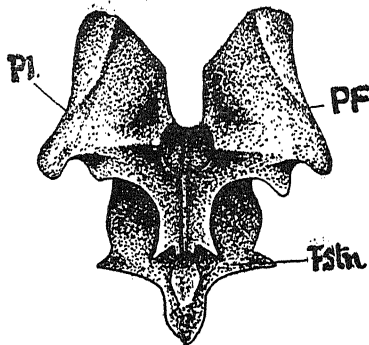

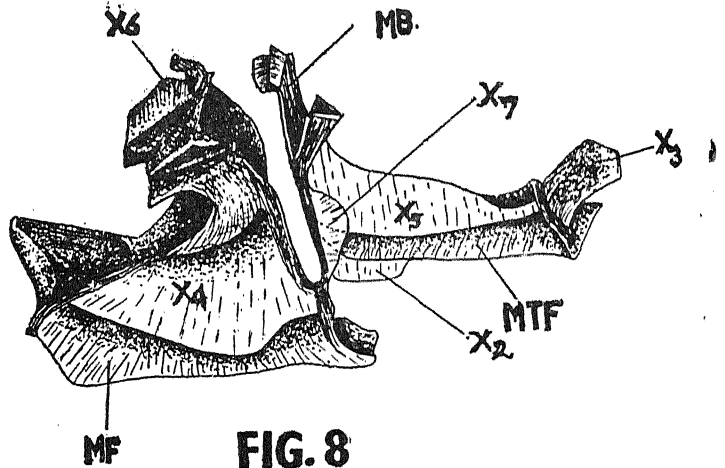FIG. 2

FIG. 3

FIG. 4

FIG. 5

FIG. 6

FIG. 7

FIG. 8

PLATE III

Fig. 1. Inner view of the eighth sternite of male.
Fig. 2. Inner view of the eighth tergite of male.
Fig. 3. Inner view of the *Lamina annularis* of male.
Fig. 4. Membranous plate under male genitalia.
Fig. 5. Ninth sternite of the male.

Fig. 6. Lateral view of the prothoracic furca dissected longitudinally.
Fig. 7. Prothoracic furca as seen from the anterior side.
Fig. 8. Lateral view of meso and meta-thoracic furca.

which form an arch in between the mesothorax and the propodeum. The posterior pair of wings is attached to its lateral extremities.

The *metatergum* (Pl. II, Fig. 1, *Mtpl.* and Pl. IV, Fig. 1.) is an oblong sclerite somewhat narrower laterad than mesad, where it carries the anterior and posterior notal processes (*anp and pnp*). It does not show any actual division into a scutum, scutellum or post-scutellum and it appears to have a median and two lateral lobes specially if seen from the ventral side. It is due to the anterior and posterior marginal phragmata being joined together by an intermediate apodemal ridge (*iar.*) on either side of the middle portion.

The *metapleuron* pleuron (Pl. IV, Fig. 2) commences from the wing base and runs obliquely downwards and backwards, to the base of the hind leg and extends to the ventral region. It does not show any division into an epimeron and episternum, but consists of two plates one upper and the other lower (*upmp and lpmp*). The dorsal end of the upper sclerite forms the base of the posterior wing, while the ventral tapers downwards and runs along the suture in between the propodeum and mesopleuron (Pl. II, Fig. 1). The lower plate (Lpmp) is broad and its posterior margin is fused with that of the propodeum. It extends to the ventral side and meets its fellow of the opposite side to form a mid-ventral suture. This can easily be mistaken for the left half of the metasternum. From the anterior portion of this suture there hangs a thin chitinous ridge (Pl. III, Fig. 7) vertically downwards which divides the right and left coxal cavities of the middle pair of legs. The ventro-posterior margin of the lower plate forms the precoxal bridge of the hind pair of legs in combination with the apophysis of the metasternum. In between the pleuron and the tergum of the metathorax, the second spiracle is situated just at the base of the hind pair of wings in the intersegmental membrane.

The meso-sternal intercoxal ridge gradually disappears near about the middle of the mid-ventral suture. Just here a thin inconspicuous line appears, which on the posterior side terminates in a small median shield-shaped plate between bases of the middle and the hind legs (Pl. III, Figs. 8, x 3.) The metasternum can be easily seen in between the coxal cavities after removing the hind pair of legs. It forms the inner border of the coxal cavities. This plate has taken more or less a vertical position and only a little portion of the posterior margin can be seen in the ventral view.

Furca—In every thoracic segment the sternum is provided with a large chitinous internal structure—the *furca*. The furcae of the prothorax and the mesothorax are very complicated while that of the metathorax is comparatively simple. The furcae of the mesothorax and metathorax lie very close to each other and consequently the mesothoracic furca is placed at the posterior and the methoracic at the anterior end of their respective segments. They are fused together at their bases.

The *prothoracic furca* (Pl. III, Figs. 7, *PF*) is an acutely triangular plate in the middle of the prothoracic space. It is attached to the sternum by an apex and a ridge which runs along the middle of the sterna. The other two apices of the triangle support two dorso-laterally concave broad plates (*Pl*) through the middle of which run the apophyses connecting the whole structure to the pleuro-sternum. These apodemal apophyses fuse with the sternum on the ventral side. The *furca* provides attachment to various muscles of this region.

The *méso-furca* (Pl. III, Fig. 8 *MTF* and Pl. II, Fig. 7 *Mtf*) which lies in the posterior part of the mesothorax, is supported on a high crest rising vertically upwards
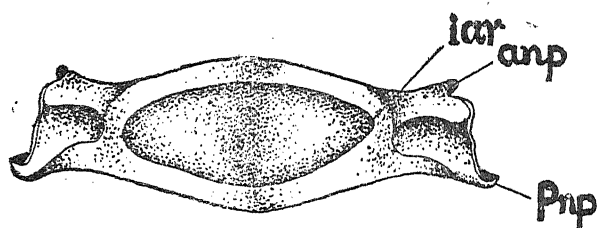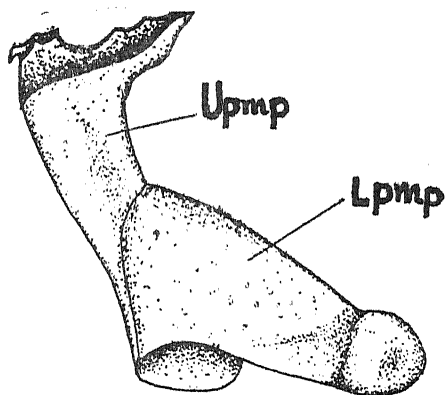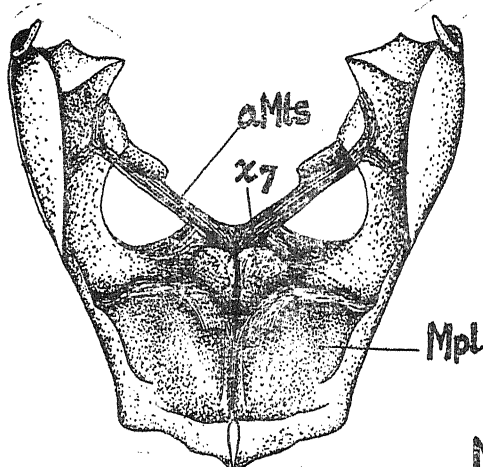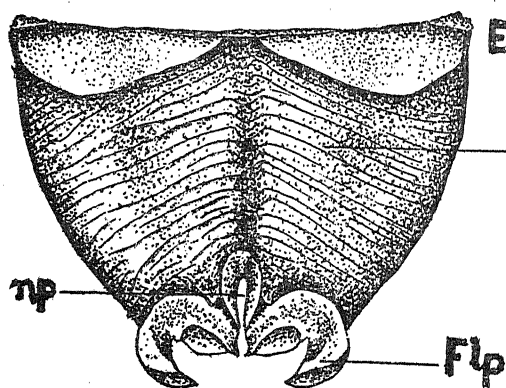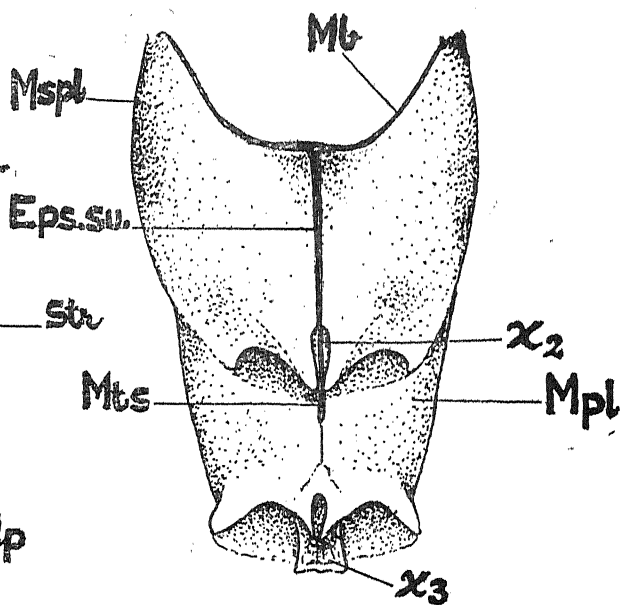
[175]

FIG. 1

FIG. 2

FIG. 3

FIG. 5

FIG. 4

PLATE IV

Fig. 1. Inner view of the metatergum.
Fig. 2. Lateral view of the metapleuron.
Fig. 3. Inner view of the metapleuron showing the metafurca *in Situ* after removing metatergum and propodeum.

Fig. 4. Ventral view of the meso and meta-thorax showing sternum of the two segments in between the pleura.
Fig. 5. A dorsal view of the propodium.

and running along the middle line. This ridge (Pl. III, Fig. 8, x 4.) gradually increases in size posteriorly and is continued into another ridge (x 5) under the metasternum. The above mentioned ridge in the mesoternum runs towards the anterior side to some length, then bifurcates and continues laterally into the antecostal phragmata. The forked ridge at its posterior end is bridged over by two broad subquadrate plates (Pl. III, Fig. 8 and Pl. II, Fig. 7, x 6) meeting together by a narrow connection mesially, and enclosing a circular space. An apodemal phragma runs dorsoventrally on either side of the subquadrate plates commencing from the ventral tip of the ridge laterad to meet the pleurites. This ridge is continued in the form of a 'U' into another shield shaped plate (Pl. III, Fig. 8 and and Pl. IV, Fig. 3, x 7) at the anterior end of the metathorax forming the furca of the metasternum. It differs from that of the bee in view of the fact that the arms of the furcae do not fuse except at the extreme ventral tip. The independent nature of each of the furca is consequently retained, but the location of the meso. furca in the posterior region of the mesothorax and of the metafurca in the anterior region of the metathorax places wasps in an important position in between the transitional and more simplified insects. This, together with other characters, throw flood of light on the evolution of Hymenoptera.

Meta-furca — Besides, the shield-shaped plate (x 7) already described, the metaformed of the metasternum bifurcating into two arms at the anterior end. These arms (Pl. IV, Fig. 3, *aMts.*) run outwards and dorsad and continue into the phragma round the anterior meta-thoracic margin.

The *first abdominal segment* is very highly modified and its tergite is called the propodeum (Pl. II, Fig. 1, *PPd*) which is a convex conical plate, sloping steeply posterad, with a deep longitudinal dark furrow mid-dorsally, from which nearly twentyfour transverse ridges (Pl. IV, Fig. 5 *Str.*) run on either side laterad. On the posterior end of the propodeum there are two processes one on each side of the longitudinal furrow lying close to each other forming a deep notch (*np*) between them through which important abdominal muscle-tendons pass into the thorax giving an additional hold fast to the abdomen. Postero-laterally the propodeum has broadly triangular emarginate flaps (*Flp*) one on each side, curving below ventrad. They extend nearly to the mid ventral line articulating the narrow anterior end of the second abdominal tergite in betweeen. The sternite (Pl. VI, Fig. 1, *lst.*) of the first abdominal segment is reduced to a very narrow and minute chitinous plate fused with the tergite of the second segment at the antero-lateral angle and also more or less with the anterior margin of the anterior petiolar process of the second sternite. In nature it is hidden by the posterior lobes of the propodeum.

Sclerites of the legs. — The *coxa* (Pl. V. Fig. 1.) is at runcate cone with the proximal margin girdled by a submarginal basicoxal suture (*Bc Su.*) forming an internal ridge and cutting off a proximal basicoxite for the attachment of the remoter and the promotor muscles. Dorsally the basicoxite is enlarged otherwise it is narrow all round. The coxa is slightly less than twice as long as broad and about half as much as the femur. The proximal end is one and a half times broader than the distal. It is articulated to the body by means of two articular condyles *i.e.*, an outer, which works against the pleuron and an inner against the sternum laterad. The external condyle is a prominent convexity over the marginal apodeme, while the inner, comparatively smaller, is a bit removed to the inner lobe of the basicoxite although lying quite adjacent to the major lobe. The coxal joint of the foreleg appears to be more or less monocondylic as the second inner condyle is very feebly developed. The coxa can, therefore, move in the anterior and posterior directions only. The
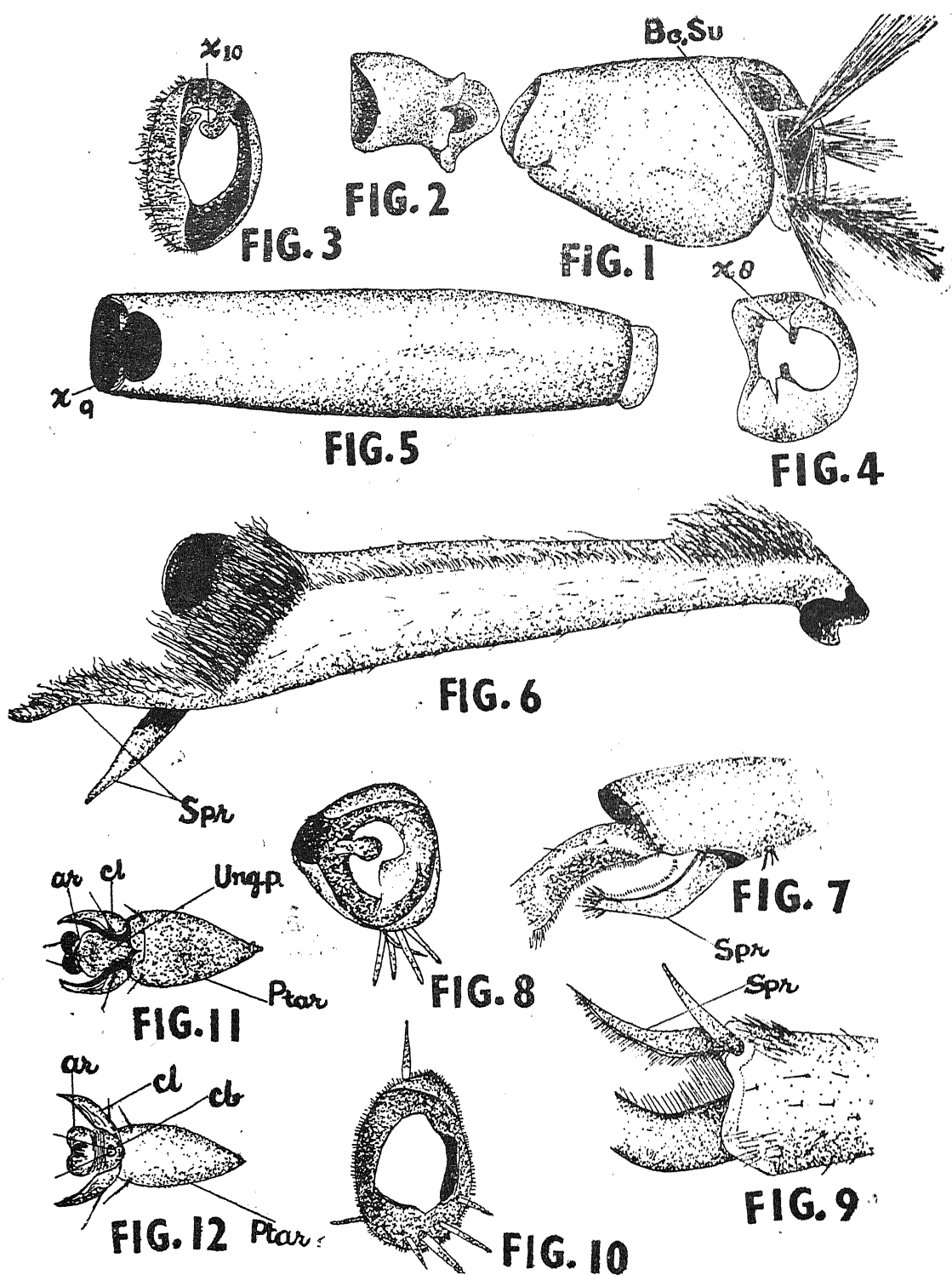
FIG. 3

FIG. 2

Bc.Su

FIG. 1

x_{10}

x_8

FIG. 4

x_9

FIG. 5

FIG. 6

Spr

ar cl   Ungp.

FIG. 8

Ptar

FIG. 11

FIG. 7

Spr

Spr

ar   cl   cb

Ptar

FIG. 12

FIG. 10

FIG. 9

PLATE V

Fig. 1   Ventral view of the middle coxa.
Fig. 2.   Dorsal view of the middle trochanter.
Fig. 3.   Ventral view of the distal end of the tibial
          cavity after removing spurs.
Fig. 4.   Inner view of the distal end of the coxal cavity.
Fig. 5.   Ventral view of femur.
Fig. 6.   Inner view of tibia.
Fig. 7.   The strigilis or antenna-cleaner on the proleg.

Fig. 8.   Inner view of the distal end of the tarsal cavity.
Fig. 9.   Toilet apparatus on the hind legs.
Fig. 10. Inner view of the proximal end of a tarsal cavity.
Fig. 11. Ventral view of the Pretarsus with adjoining
          appendages.
Fig. 12. Dorsal view of the pretarsus with adjoining
          appendages.

[179]

distal end of the coxa bears two closely adjoining processes, on the anterior and posterior margins. Adjacent to these processes, are facets (Pl. V, Fig. 4, z 8) projecting into the trochanterocoxal cavity for the articulation with the trochanter. In the coxa of the foreleg there is a very prominent outwardly directed process on the ventral side of the distal end. There is also a deep notch in the outer distal margin of the coxa to allow a greater play to the trochanter.

The *Trochanter* (Pl. V, Fig. 2.) is small, three times as long as broad, subconical; dorsal margin obtuse and shorter, the ventral proximal end slightly broadened into a dorsal round process and two sharp lateral ones, under each one of which there is a notch for the muscle attachments. The joint allows the movement of the leg up and down only as the hinge is horizontal. The distal end forms a more or less flexed joint to the base of the femur, and is slightly carinate on the ventral side. The trochanter of the foreleg has only one stout process directed anteriorly inwards at the proximal end. On the distal margin there are two factes within the trochanteral cavity very prominent in the middle and hind legs, one at the dorsal and the other at the antero-lateral margin.

Femur (Pl. V, Fig. 5.) is long, stout, a little less than twice the length of the coxa, five times longer than broad, and tapering at both the ends. The femur of the foreleg is somewhat curved ventrad, and is the shortest ; the hind femur longest. The middle one is as much longer than the femur of the foreleg as it is shorter than that of the hind one. The proximal end of the femur has two facets for articulation with the distal end of the trochanter ; it also has two processes (x 9) directed into the femoral cavity. They originate laterally on the distal margin affording a horizontal joint. The ventral margin of the distal end has a deep notch for allowing greater freedom of movement for the rest of the leg in a vertical plane.

Tibia (Fig. 6.)—is a long slender conical segment of the leg nearly as long as the femur, six times longer than its greatest width, tapering proximally, with the ventral surface, more or less flat. At the proximal end there are two sharp lateral processes and a round dorsal process similar to those found in the trochanter. The distal end has an oblique apodemal ridge and a round process (x 10) directed into the tibio-tarsal cavity for the articulation of the tarsi. Tne margins are notched anteriorly and posteriorly for free-movement of the tarsi. Besides being clothed by fine pubescence, it has a frill of long setae all along the inner ventral line but the tibia of the hind leg has a small tuft of very long dark brown setae at the proximal end and a bigger tuft at the distal end.

Strigilis—(toilet apparatus for cleaning antennae) is provided in the tibia of the foreleg as a long slightly inwardly curved spur at its inner distal margin (Fig. 7). It has a transparent chitinous comb with fine setae at the free margin and a tuft of long fine brown setae at the tip of the spur. The portion of the tarsus lying against the spur has an oval concave facet-like apodemal ridge with setae growing thickly over its broad inner margin. The antennae are passed through between the spur and the tibial facet, whose anterior border further has a sharp carina, which cleans them with the help of the setae on its border and the spur. The head and prothorax are also brushed by the frill of setae on the inner margin. In order to clean the legs they are also rubbed against each other. The toilet apparatus over the hind legs (Pl. V, Fig. 9) is formed by the spurs found on the inner ventral side of the tibia. One of them is longer, slightly bent inwards and provided with setae on the inner margin. The slightly concave surface of the tarsus lying just opposite to it has specially long setae along the inner margin. Wings and legs are passed through the axil
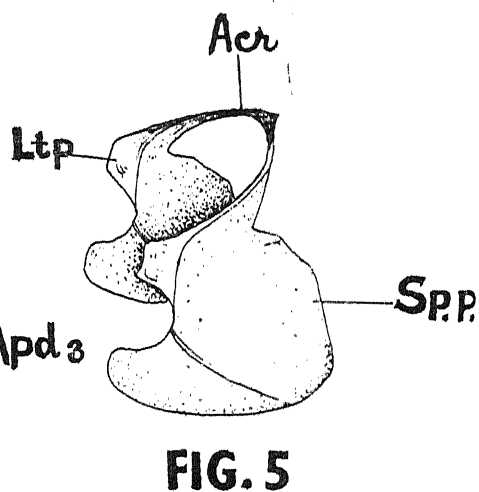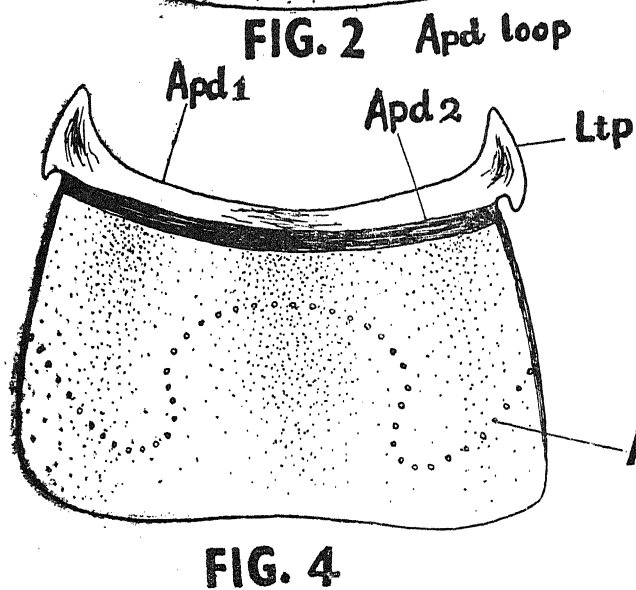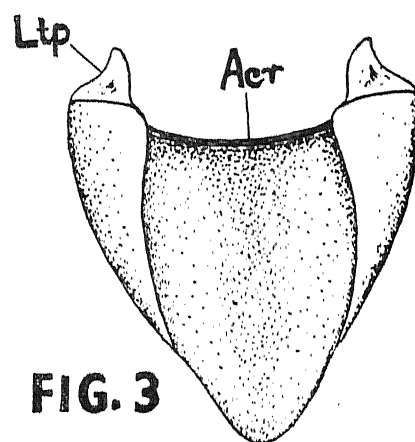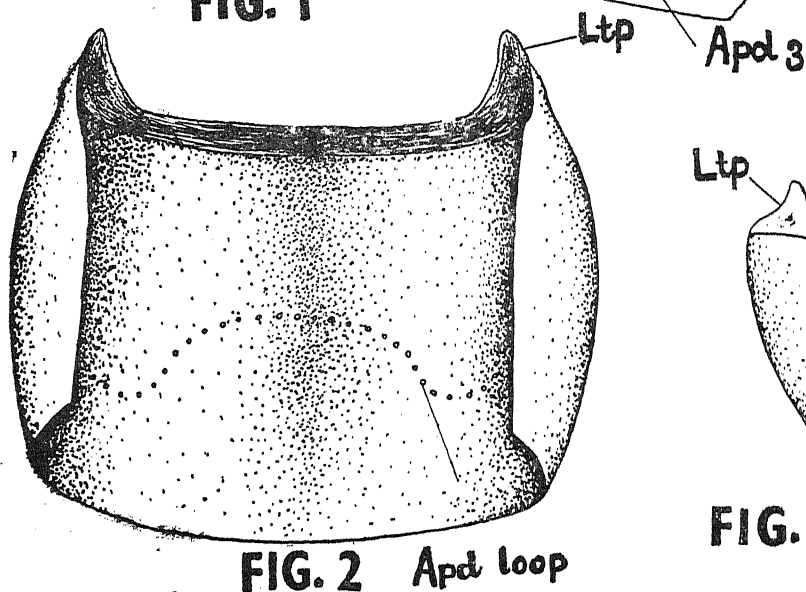
[179]

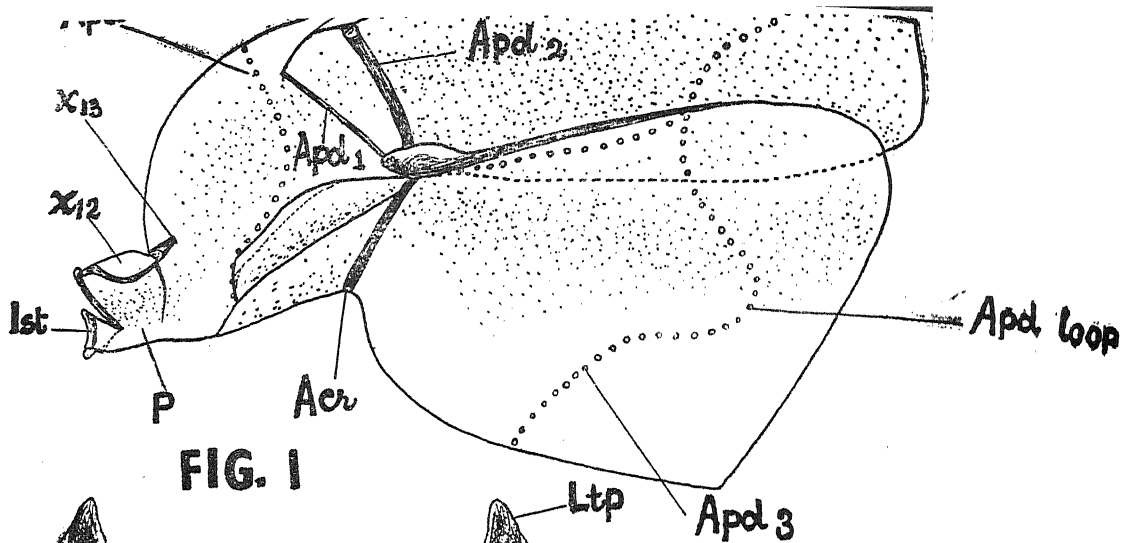**FIG. 1**

FIG. 2

FIG. 3

FIG. 4

FIG. 5

PLATE VI

Fig. 1. Inner lateral view of the second and third abdominal segments.
Fig. 2. Inner view of the third abdominal tergum.
Fig. 3. Inner view of the telson of the female wasp.
Fig. 4. Inner view of the third abdominal sternum.

Fig. 5. Dorso-lateral view of the eighth abdominal tergum showing its reduction dorsally and the lateral portions of the sclerite forming the spiracular plates.

between the spur and the tarsi and cleaned by the setae which serve as a brush. The fringe of setae all along the inner margin of the tibia and tarsi also brushes the abdomen, thorax, upper surface of the wings and the legs. The spurs on the middle legs are not supplied with setae but the tarsi and tibia have the frill of setae on the inner margin but no tufts on the proximal or distal ends. They partly help in the toilet of the abdomen and the wings.

Tarsus of the leg is subdivided into five tarsomeres jointed movably to each other. The basitarsus is a long, slender, cylindrical structure slightly tapering proximally and buldging distally, slightly shorter than the femur, nearly nine times as long as broad. The hind basi-tarsus has a fringe of long setae along the inner ventral line from the proximal to the distal ends forming a part of the toilet apparatus. The whole surface is covered by fine setae with scattered stout erect spines. The proximal end of the basitarsus is provided with a process and an oblique ridge in its cavity (Fig. 8.) where its main muscles are inserted. Its distal end has facets for articulation with the other tarsi. The distal margin is deeply notched dorsally and ventrally to allow greater movement to the adjacent tarsi but in a horizontal plane only. The lateral margins are supplied with a row of five to eight long stout peg-like bristles just guarding the tarsal cavity. The following four tarsi are gradually reduced in size and have a tendency to be depressed distally and tapering into a round apex proximally, slightly convex dorsally and concave-ventrad. The lateral margins of the distal ends are produced forward to form a notch on the dorsal and on the ventral sides. They are provided with six to eight hard bristles. The pretarsus, although similar in shape as the preceding parts, is comparatively a little longer, nearly one and a half times as long and twice as broad. It bears a pair of highly chitinised dark brown, ventrally curved movable claws (Fig. 11 and 12, *cl.*) atached to the distal end of the pretarsus by means of two knob-like condyles projecting into the articulating pretarsal cavity from its dorsal plate. Each claw is provided with a long ventral setae. On the lateral side of the distal margin of the pretarsus there is a pair of stout setae projecting over the articulating joint. The pretarsus is ventrally connected to a small plate—the unguitractor plate (Pl. V, Fig. 11, *Ung. p.*—Snodgrass) by means of a membrane. At the proximal end of this plate is inserted the long apophysis which originates in the trochanter and passes through the femur, tibia and tarsi. This plate is continued into a median thick lobe, the arolium (Pl. V, Fig. 12, *ar.*) lodged between the two claws. The dorsal surface is covered over by a dark chitinous membrane and is provided with a large number of minute lamellae, which, while the insect walks, create partial vacuum and thus enable it to cling to any smooth surface. In a transverse section of the arolium, large vacant spaces appear under the chitinous layer. These gaps help in providing vacuum, necessary for clinging. The arolium appears to be a thick pad as if folded twice over on the distal side and it partially opens owing to the pressure it receives due to the weight of the body. Just proximal to the arolium, there is a chitinous bar (*cb.*) raised vertically on the dorsal side. At its upper end it has two long stout setae projecting distally over the folded arolium. The claws, chitinous plate, and the other adjacent parts are sparsely clothed with fine setae.

The Abdomen.—The tergites curve round laterally reaching almost to the ventral side, thus occupying nearly three-fourth of the abdominal diameter, the rest is covered over by the sternites. Although the abdomen actually consists of eight tergites and sternites in the male and seven in the female excluding the propodeum, yet only seven tergites, and sternites are visible in the male and six in the female ; the rest have been telescoped and concealed within the posterior terminal sclerites. The posterior margin of each tergite overlaps the anterior of the following and also

[181]

the lateral margin of the corresponding sternite. Similarly the posterior margin of each sternite also overlaps the anterior of the following.

The first tergite has already been discussed in connection with the thorax, to which it has closely, associated itself as propodeum. The corresponding sternite, described already, forms the anterior ventral margin of the petiole.

The second tergite or the first apparent one is nearly one fourth of the whole abdominal length and is about as long as broad posteriorly. It is a sub-triangular dome-shaped plate (Pl. VI, Fig. 1.) with its apex prolonged anteriorly, forming a narrow tube, the petiole (*p.*) behind which it is abruptly convex. Ventrally on either side, the tergite is strongly retracted cephalad. There is a prominent crest (*x* 12) running longitudinally along the dorsal surface of the petiole. The crest is higher at the anterior end. The anterior margin of the petiole is highly chitinised and slightly prolonged mesially and also latero-ventrally into two irregular lobes at the bases of which there is a notch on each side. The tendons of muscles pass through these notches. The posterior margin is also convex mesially. The sternite is more or less a flat subtriangular plate with the apex produced anteriorly into a trumpet-shaped process forming the floor of the petiole. Its anterior margin is rounded and the posterior straight. The anterior lobe of the sternite is immovably fused with the corresponding tergite in the petiolar region but the main body of the sternite is free and the tergite overlaps its edges. A curved sharp angle exists in between the broad main and petiolar lobe of the sternite, and the surface of the former is slightly pleated transversely. The posterior margin is strongly ribbed transversely more or less fused with the anterior antecosta of the third sternite which is sharply bent ventrad. Internally the second tergite, just at the constricted anterior part, has a transverse heavily chitinised rod with two short anterior processes directed forwards for a very short distance along the roof of the petiole. (Pl. VI, Fig. 1, *x* 13.). To this rod in between the processes is attached the thick tendon of the muscle which passes through the two median posterior processes of the propodeum. The inner surface of the tergum has an apodeme (*apd.*) running transversely up to the base of the petiole.

The third abdominal segment, apparently the second, is the longest and broadest of all. Its tergum covers slightly more than one fourth of the whole length of the segment. Anteriorly it has a highly chitinised antecostal apodemal ridge (*apd* 1). There is, besides, another more conspicuous broader apodemal ridge (*apd* 2.) posteriorly, parallel to the first, and meeting it laterally. This affords attachment to lateral intersegmental muscles. The posterior margin overlaps the anterior of the next and its own anterior margin is telescoped under the second abdominal tergite. An apodemal ridge (*apd* 3) runs transversely parallel to the posterior margin but this ridge is thrown into a loop on either lateral sides and these act as important places for muscle attachment. To this apodemal line with loops, is attached the intersegmental membrane connecting this sternite to the next posterior one. The third sternite is about three fourth the length of the tergite, comparatively narrow at the anterior margin, where it has a thick apodemal intercostal ridge (*Acr*) fused with the anterior margin of the second sternite. Besides, there is a broad triangular apodemal process, on either antero-lateral angle at the end of the above ridge. The lateral margins are also chitinised and carry muscles. An apodemal ridge also runs along its second-third transversely with the loops as in the tergum.

The sclerites of the rest of the segments posteriorly are similar to these except in their gradually descreasing size. The posterior margin of the sternites from the

[182]

third onwards in the male, is increasingly concave until the sixth is distinctly truncate in the middle. All the sclerites, dorsal or ventral have a narrow and a broad apodeme running parallel with lateral triangular processes (Pl. VI, Fig. 2 and 4, *Ltp.*) at the anterior margin and a looped apodeme at the posterior third, the later being absent in the terminal sclerites.

The telson in male *i.e.* the tergite of the 8th abdominal segment is a triangular plate rounded at the posterior apex (Pl. III, Fig. 2). Anteriorly it has a thick chitinous antecostal ridge (*acr*) with the usual antero-lateral triangular processes. The sternite corresponding to this segment is also triangular but has two claspers (Pl. III, Fig. 1. *Clsp.*) arising postero-laterally. In the mid-ventral line of this sclerite there is a forwardly directed chitinous spine (*Apr*) which is prolonged forward into a stout process. The apodemal ridge where the intersegmental membrane from a posterior segment attaches itself, is absent. The lateral margins are continued into long flap-like processes and these may be easily mistaken for the vestigeals of the next tergites but since they afford insertion to the muscles originating from the telson, these should be considered as the processes of the sternite, which corresponds to the telson *i.e.* the eighth sternite.

In the females also, the telson *i.e.* the tergite of the seventh segment apparently of the sixth, (Pl. VI, Fig. 3.) is triangular but the posterior end, is relatively sharper. The same is true of the sternite but it is not as far prolonged as the telson and is left a little behind. It is provided with a tuft of long setae ventrally a little behind the anterior antecosta. The eighth tergite of the female is completely hidden under the telson. It is reduced to broad subtriangular flaps, one on either side. These are called *spiracular plates*, and are interconnected antero-dorsally by a narrow chitinous band, which has an apodemal apophysis anteriorly (Pl. VI, Fig. 5). The eighth sternite is a quadrate plate on either side, interconnected with each other, nearly three fourth of which is covered over by a spiracular plate of the tergite and in turn covers partly the ninth sternite. It is ventrally produced into the characteristic sheath of the stylets. The remaining sclerites form part of the external genital apparatus.

<div align="center">KEY TO LETTERING</div>

Abd.	.. Abdomen.	At.	.. Muscles for side-ways
Acr.	.. Anticostal ridge.		movement of the
Ad.	.. Depresser of antenna.		antenna.
Aeps.	.. Anepisternum.	Bc. Su.	.. Basicoxal suture.
Al.	.. Levator of the antenna.	Cb.	.. Chitinous bar.
Amts.	.. Arms of metasternite	Cl.	.. Claw.
	apodeme.	Clsp.	.. Clasper.
Apd. 1, 2, 3. .	Various apodemes.	Cly.	.. Clypeus
Apdloop.	.. Apodeme for attachment	Cx.	.. Coxa.
	of Intersegmental	Ep.	.. Episternum.
	membrane.	Eps. Su.	.. Episternal suture.
Apr.	.. Anterior process of the	Ep. Su.	.. Epicranial suture.
	eighth sternite of male.	Esu.	.. Epistomal suture.
Ar.	.. Arolium.	Ey.	.. Eyes

<div align="center">[183]</div>

F.   .. Femur.

Flp.   .. Flaps or process of the propodeum.

Fr.   .. Frons.

Ge.   .. Gena.

Ge. Su.   .. Genoclypeal suture.

Gf.   .. Gular fossa.

Hst.   .. Hypostomal bridge.

iar.   .. Intermediate apodemal ridge between the lateral and median lobes of the meta thorax.

Ist.   .. First abdominal sternite.

Lpmp.   .. Lower plate of the meta-pleuron.

Lpr.   .. Lateral process of the eighth sternite.

Ltp.   .. Lateral traingular process of tergites and sternites.

Mb.   .. Marginal band of meso-sternum.

Md.   .. Mandible.

MF.   .. Mesofurca.

Mpl.   .. Meta pleuron.

Ms.   .. Mesoscutum.

Msct   .. Mesoscutellum.

Mspl.   .. Mesopleuron.

Ms. T.   .. Meso-thorox.

Mtf.   .. Metafurca.

Mtpl.   .. Metapleuron.

Mts.   .. Meta sternum.

Mtt.   .. Metatergum.

Mt. T.   .. Meta thorax.

n.   .. Notch at posterior end of propodeum.

Np.   .. Notch between which Abdominal muscle tendons pass to the thorax.

Nr.   .. Notal ridge of the meso-scutellum.

Oc.   .. Ocelli.

Ocp.   .. Occipital plate.

Ocp. F.   .. Occipital foramen.

Oc. Su.   .. Occipital suture.

P.   .. Petiole.

PF.   .. Profurca.

Pl.   .. Pleat of the profurca.

Po. St.   .. Post scutellum.

Po. Su.   .. Post occipital suture.

Ppd.   .. Propodeum.

Ppl.   .. Propleuron.

Psc.   .. Proscutum.

Pst.   .. Proscutellum.

Pstn.   .. Prosternum.

Ptar.   .. Pretarsus.

Prt.   .. Prothorax.

Scr.   .. Inter-scrobal ridge.

Sct.   .. Scutellum of Prothorax.

Sg. Su.   .. Subgenal suture.

Spp.   .. Spiracular plate.

Spr.   .. Spur.

Stn.   .. Sternite

Str.   .. Striations of the propodeum.

T.   .. Tegula.

Tar.   .. Tarsi

Ten.   .. Tentorium.

Ten. cf.   .. Clypeo-frontal branch of tentorium longitudinlle

Tenl.   .. Tentorium longitudinale.

Ten. la.   .. Tentorium longitudinal antennalis.

Ten. Ocp. .. Tentorium Occipital.

Tent.   .. Tentorium transversal.

Tr.   .. Trochanter.

Ung p.   .. Unguitractor plate.

Upmp.   .. Upper plate of Meta-pleuron.

X.   .. The process of the Tentorium transversal.

X1.   .. The process of the gular wall.

X2.   .. Chitinous ridge in between middle coxal cavity.

X3.   .. The metasternal plate in between the hind coxal cavity.

X4.   .. The ridge under the mesofurca.

X5.   .. The ridge under the metafurca.

X6.   .. The subganglionic plate of the mesofurca.

X7.   .. The Shield-shaped plate at the anterior end of the metafurca.

X8.   .. Facets at the distal end of the coxal cavity for articulation with Trochanter.

X9.   .. Processes at the distal end of the femoral cavity.

X10.   .. A round process at the distal end of the tibial cavity.

X11.   .. Knoblike processes of the pretarsus to articulate with claws.

X12.   .. Crest over the petiole.

X13.   .. The transverse chitinous rod over the seconp tergite.

BIBLIOGRAPHY

Allen, H. W. and H. A. Jaynes., 1929. Contribution to the taxonomy of Asiatic wasps of the genus—*Tiphia*. *Proc. U. S. Nat. Mus.*, 76, Art. 17 : 1-105.

Berlese, A., 1909. Gli Insetti, Vols. I and II, *Societa Editrice Libraria*, 243 and 305, figs. 279 and 352.

Carpenter, G. H., Insects : Their structure and life.

Comstock, J. H., 1936. An introduction to Entomology, Ed. 8, Comstock Publishing Company, Inc. New York.

Crampton, G. C., 1909. A contribution to the Comparative Morphology of the thoracic sklerites of Insects. *Proc. Acad. Nat. Sciences*, Philadelphia.

. . . 1914. The ground plan of the typical Thoracic Segment in winged Insects, *Zool. Anz.*, 44 : 56-57.

. . . 1919. A phylogenetic study of the mesothoracic tergaʃ and wing bases in Hymenoptera, Neuroptera, Mecoptera, Diptera, Trichoptera and Lepidoptera. *Psyche*, 26 (9) : 58-64, pls. 1 and 2.

. . . 1920. Remarks on the basic plan of the terminal abdominal structures of the males of winged insects. *Can. Entomol.*, 52 : 178-183.

Eschscholtz, J. F., 1920. Beschreibung des inneren Skeletts eineger Insekten aus verschiedenen Ordnungen. *Beitr. Z. Natur Kunde aus den Ostscaprovinzen Russlands*. Dorpat. pp. 24-49. Con. 2.

Imms, A. D., 1938. A general text-book of Entomology. Ed. IV. Methuen & Co. Ltd., London.

Kleuker, F., 1883. Über endoskelettale Bildungen bei Insekten. *Dissert. Gottingen Universiätt*, p. 53.

Nelson, J. A,. 1918. The segmentation of the abdomen of the honeybee. *Ann. Ent. Soc. Amer.*, 11 : 1-8, 2 Fig. pl. I.

Saunders, E., 1884. Further notes on the terminal segments of aculeate, Hymenoptera. *Trans. Ent. Soc., London*, 251-267.

Snodgrass, R. E., 1909. The thorax of insects and articulations of wing. *Proc. U. S. Nat. Mus.*, 36 : 511-595, pls. 40-69.

. . . 1927. Morphology and Mechanism of the Insect thorax. *Smith. Misc. Coll.* 80 (1)-180.

. . . 1928. Morphology and evolution of the insect head and its appendages. *Smith. Misc. Coll.*, 81 (3) : 158.

. . . 1931. Morphology of Insect abdomen. Part I, General structure of the abdomen and its appendages. *Smith. Misc. Coll.*, 85(6) : 1-128.

. . . 1935. Principles of Insect Morphology. Mc Graw-Hill Book Co., Ltd.

CONTENTS